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METABOLISM AND PHARMACOKINETICS

OF ^{14}C-WR 178,460.HCl

IN THE DOG

Study Report No. 2

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We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

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To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulations as set forth in 'Title 21 of the US Code of Federal Regulations, Part 58', with the exception of possible minor items, none of which is considered to have an impact on the validity of the data or the interpretation of the results in the report.

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This report has been audited by HRC Quality Assurance Unit and is considered to be an accurate presentation of the data produced during the course of the study.

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Following administration of a single oral dose of ^{14}C-WR 178,460.HCl to a dog at a level of 21 mg/kg most of the radioactivity was excreted in the faeces 72.4% during the first 24 hours and a total of 97.2% during 7 days. Only 0.1% of the dose was excreted in the urine during 7 days.

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Plasma concentrations of radioactivity reached a plateau at 2 hours and remained within the range 0.58 to 0.73 µg equiv./ml until 2 days. Concentrations then appeared to decline biphasically with with an initial half-life of about 53 hours. At 21 days, the concentration had declined to 0.02 µg eqiv./ml.

Peak plasma concentrations of unchanged WR 178,460 occurred at 4 to 5 hours (0.4 µg/ml) and then declined with a half-life of about 20 hours.

Most of the radioactivity in the 0-24 hour faeces sample was associated with unchanged WR 178,460 but in later samples chromatographically more polar components predominated.

Project Title: Metabolism and Pharmacokinetics of ^{14}C WR 178 460 Hydrochloride in the Dog.

Project No.: WRI 2

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This work was conducted between January 14 1983 and February 18 1983. The experimental work was supervised directly by Dr. David Kirkpatrick, Senior Scientist, assisted by Christina Finn with the technical assistance of David Roberts, Chief Animal technician.

In conducting the research described in this report the investigators complied with the Home Office regulations governed by the Cruelty to Animals Act, 1876 Great Britain and the "Guide for the Care and Use of Laboratory Animals" DHEW, NIH Publication No. 80-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

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SUMMARY

1. The purpose of this study was to carry out a pilot investigation of the metabolism and pharmacokinetics in the beagle dog, of the compound ^{14}C-WR 178,460.HCl, a potential pharmacologically active metabolite of the anti-malarial drug WR 171,669.HCl. ^{14}C-WR 178,460.HCl was administered orally, as a suspension in 1% aqueous carboxymethyl-cellulose solution, to a single dog at a dose level of 21 mg/kg.

2. Excretion of radioactivity in faeces accounted for 72.4% dose during the first 24 hours after dosing and for 97.2% dose in seven days. A total of only 0.1% dose was excreted in urine.

3. Plasma concentrations of radioactivity reached a plateau at 2 hours and remained within the range 0.58 to 0.73 µg equivalents WR 178,460 free base/ml (µg/ml) until 2 days. Concentrations then appeared to decline biphasically with an initial more rapid phase ($t_{\frac{1}{2}}$ 52.6 h ± 9.5 h S.E.) followed by a slower terminal phase for which a half-life could not be precisely determined. By 21 days the plasma concentration had declined to 0.02 µg equiv./ml.

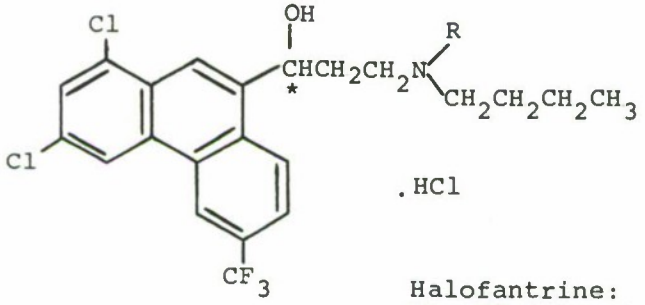
4. Whole-blood concentrations of radioactivity were slightly higher than the corresponding plasma concentrations during the period from 5 to 24 hours after dosing (maximum blood : plasma ratio 1.15) but then declined more rapidly to be below 0.06 µg/ml at 8 days.

5. Peak plasma concentrations of unchanged WR 178,460 free base occurred at 4 to 5 hours after dosing (0.40 μg/ml) at which times WR 178,460 represented about 60% of total plasma radioactivity. Concentrations then declined to be below the limit of detection (0.04 μg/ml) at 72 hours. The plasma concentration-time results were fitted to double exponential curves which indicated that the half-life for the decline in concentrations was in the range 19 to 21 hours. The remainder of plasma radioactivity was chiefly associated with components eluting close to the solvent front under the reverse-phase hplc conditions used.

6. Most of the radioactivity (86%) in solvent extracts of the 0 - 24 hours faeces sample was associated with unchanged WR 178,460. In extracts of faeces from 24 - 72 hours, radioactivity was mainly associated with components eluting close to the solvent front under the reverse-phase hplc conditions used.

INTRODUCTION

The compound WR 178,460.HCl is a potential, pharmacologically active, metabolite of the new anti-malarial drug halofantrine (WR 171,669.HCl) In order to provide a comparison between the two compounds and to obtain further information which might be of help in the elucidation of the metabolic fate of halofantrine, a study has been conducted into the metabolism of ^{14}C-WR 178,460.HCl after oral administration to a beagle dog. This report describes the results obtained in this study. The results of a pilot study with ^{14}C-halofantrine in the dog have been described in Study Report No. 1 and the results of a full study with ^{14}C-halofantrine in the dog, together with a comparison of the two compounds will be the subject of a future report.



Halofantrine: R = $CH_2CH_2CH_2CH_3$
WR 178,460.HCl: R = H

* Denotes position of ^{14}C-label

EXPERIMENTAL

Materials

^{14}C-WR 178,460.HCl (Lot No. 3793-91), of stated specific activity 16.0 Ci/mol, was supplied by Research Triangle Institute, Post Office Box 12194, Research Triangle Park, North Carolina 27709, U.S.A. Radiochemical purity was determined by thin-layer chromatography in two solvent systems, followed by quantitation of radioactivity on silica gel by liquid scintillation counting, as described in the "Methods" section. Radiochemical purities in the two solvent systems were:

System A: 96%, System B: 98% (Appendix 1)

Non-radioactive WR 178,460.HCl (Lot A1, BK 21070) was supplied by Walter Reed Army Institute of Research, Washington, D.C. 20307, U.S.A. Solvents and other reagents used were obtained from suppliers in the United Kingdom and were of analytical grade or equivalent quality.

Animal experiments

One adult male beagle dog weighing 12.5 kg at the time of dosing, was obtained from the Department of Dog Toxicology, Huntingdon Research Centre plc, Huntingdon, U.K. (Toxicology Departmental number J41 G, Litter number 2DS6). The animal was given access to food (SQC Laboratory Diet A, Batch No. 1726, Special Diets Services Limited, Witham, U.K.) and water *ad libitum*, with the exception of the period between 16 hours before and 4 hours after dosing, when food was withdrawn from the cage. The health status of the animal had been approved after examination by a veterinary officer.

For preparation of the dose, (Appendix 2), ^{14}C-WR 178,460.HCl (6.01 mg) and non-radioactive WR 178,460.HCl (268.7 mg) were mixed together in solution in methanol (10 ml). Aliquots (5 μl) of this solution were removed for measurement of radioactivity. The methanol was evaporated in a stream of nitrogen to yield a fine dry white powder. This diluted ^{14}C-WR 178,460.HCl equivalent to a nominal dose level of 20 mg/kg, was then suspended (using a Soniprobe Type 7530A) in a total of 14 ml of 1% (w/v) aqueous sodium carboxymethylcellulose. The dose suspension was immediately administered to the animal *via* a gavage tube into the stomach. The dose syringe and tubes were washed through with a further 20 ml of dose vehicle. The dose container, syringe and stomach tube were thoroughly washed with methanol and the radioactivity content of these washings was measured. After subtraction of radioactivity in dose washings the net dose to the animal was 265 mg, equivalent to a dose level of 21.2 mg/kg bodyweight.

Following dosing the animal was housed in a stainless steel metabolism cage designed to facilitate the separate collection of urine and faeces. Urine was collected, into receivers cooled with solid carbon dioxide, during 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 hours after dosing. Faeces were collected during 24-hour intervals up to 168 hours after dosing. The interior of the cage was washed with water after every 24-hour sample collection period.

Blood samples (ca. 10 ml) were withdrawn from the cephalic vein into heparinised tubes, immediately prior to dosing and at 15, 30 minutes, 1, 2, 3, 4, 5, 6, 7, 12, 24, 30 hours and 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 18 and 21 days after dosing. From each sample a 1.5 ml aliquot was removed for measurement of radioactivity in whole-blood. The remainder was centrifuged and the plasma separated from the cells.

All samples were stored at -20°C until taken for analysis.

Methods

Measurement of radioactivity

Radioactivity was measured by liquid scintillation counting using either Philips (Models PW 4510 and 4700, Philips N.V., Eindhoven, Holland) or Intertechnique (Model SL 4200, Intertechnique Limited, Portslade, Sussex, U.K.) liquid scintillation counters. After choosing the optimal channel settings, quench correction curves by the sample channels ratio and external standard channels ratio methods were prepared from radiochemical standards (^{14}C-hexadecane, Amersham International p.l.c., Amersham, U.K.). The coefficients of a quadratic quench curve function were calculated and entered in the analyser data processors which automatically calculated disintegration rates. The validity of the calibration curves was checked at intervals of approximately 2 weeks. Samples were counted for 4 minutes with the exception of plasma and whole-blood samples which were counted for 10 minutes. Radioactivity in amounts less than twice background levels was considered to be below the limit of accurate measurement. With equal counting times for both background and sample, the maximum statistical counting error at this limit was about 10% of the net count rate. An exception to this was the measurement of radioactivity in fractions of hplc eluate obtained after chromatography of plasma extracts when the limit of accurate measurement was taken as 16 dpm above a background of 44 dpm giving a statistical counting error of 20%.

Preparation of samples

Faeces were extracted once by homogenisation with methanol and twice further by homogenisation with methanol : diethylamine (9 : 1, v/v). After centrifugation, radioactivity was measured in all extracts and in the residue remaining after the third extract. Samples of urine, cage washings, plasma, solvent extracts of faeces and solutions associated with dose measurement, were mixed with MI 31 scintillator (Packard Instrument Co. Ltd., Reading, U.K.) for liquid scintillation counting. Samples of whole-blood and faecal residues were mixed with dry cellulose powder and burned in oxygen using an Automatic Sample Oxidiser (Model 306 Mk 2 Tri-Carb ®, Packard Instrument Co. Ltd.). The combustion products were absorbed into Carbosorb ™ and mixed with Permafluor ®V Scintillator system. Recoveries of radioactivity from carbon-14 standards for sample oxidisers (Amersham International p.l.c.) burned in the oxidiser exceeded 99%. Measurements of radioactivity were not corrected for oxidiser efficiency.

Thin layer chromatography (tlc) for the determination of radiochemical purity

Tlc was carried out on pre-layered glass-backed Kieselgel F254 plates (E. Merck. AG., Darmstadt, Germany) of layer thickness 0.25 mm.

The developing solvent systems were:

A. Chloroform : methanol : aqueous ammonia (35%, w/v) (90:10:1, v/v)

B. Tetrahydrofuran : acetic acid (1:1, v/v).

Radioactive components on thin-layer plates were detected by apposition autoradiography, using Singul X RP X-ray film (Ceaverken AB., Strängnäs Sweden). For quantitation, areas of silica gel were scraped from the plates, water (4 ml) was added and the samples placed in an ultra-sonic bath for 5 minutes. Radioactivity was measured by liquid scintillation counting after adding Scintillator T (10 ml, Fisons Scientific Apparatus Ltd., Loughborough, U.K.). Radioscans were obtained using a Berthold thin-layer scanner (Model LB 2722).

High performance liquid chromatography (hplc)

Hplc was carried out using a Waters Model AL 202, high performance liquid chromatograph (Pump M6000A, Injector U6-K, Waters Associates Ltd., Stockport, U.K.) with a variable wavelength U.V. detector (Model LC 55 Perkin-Elmer Ltd., Beaconsfield, U.K.) set at 309 nm. A Zorbax C_8 reverse phase column, of mean particle diameter 7µ, length 25 cm and internal diameter 0.46 cm, was used (Du Pont Company, Analytical Instruments Division, Wilmington, DE 19898, U.S.A.). The mobile phase was methanol: 0.05M aqueous sodium acetate (85 : 15, v/v) containing 1% (w/v) sodium lauryl sulphate and adjusted to a pH of 6.5. The flow rate was 2 ml/min. Under these conditions WR 178,460.HCl had a retention time of 11-12 minutes.

Hplc of faecal extracts

Aliquots (5 ml) of the first (methanol) extracts of faeces collected during 0-24, 24-48 and 48-72 hours after dosing were mixed with 1 ml of a 6% aqueous solution of sodium lauryl sulphate. These samples were centrifuged at 180,000 'g' and 4°C for 30 minutes and aliquots (100 µl or 200 µl) of the supernatant were injected onto the hplc. One minute fractions of column eluate were collected and their radioactivity content measured by liquid scintillation counting after addition of MI 31 scintillator (7 ml). Portions of the second and third faecal extracts (methanol : diethylamine 9 : 1, v/v) from the above time periods were pooled in proportion to the total sample volume. Aliquots (5 ml) of the mixtures were evaporated to dryness on a rotary-film evaporator at 30°C and redissolved in mobile phase. These solutions were then ultracentrifuged and analysed as described above.

Hplc of plasma extracts

To 1 ml of plasma was added 4 ml of a 1% (w/v) solution of sodium lauryl sulphate in methanol. The precipitated protein was centrifuged (ca. 2000 'g') and the supernatant extract removed. This extract was cooled to 4°C and centrifuged at ca. 180,000 'g' for 30 minutes. Aliquots (2 ml) of the supernatant were subjected to hplc as described above. The overall recovery of authentic ^{14}C-WR 178,460.HCl, from spiked dog plasma, by this method was about 94%.

Mass spectrometry

Mass spectra were recorded on a Micromass 16F mass spectrometer (V.G. Analytical Ltd., Altrincham, U.K.) linked to a VG Display Digispec on-line data system based on a PDP 8a computer. Samples were introduced via the direct insertion probe. Electron impact mass spectra were recorded at an electron beam energy of 70 e.v., a trap current of 100 μA and a source temperature of 200°C.

Statistical methodology

Two curves were used as models for fitting concentration-time curves to the data obtained in this study. These are the double exponential (WR 178,460 free base concentrations),

$$c = A\,(e^{-\lambda_2(t-\tau)} - e^{-\lambda_1(t-\tau)})$$

and the triple exponential (total radioactivity concentrations),

$$c = -(A_1 + A_2)e^{-\lambda_1(t-\tau)} + A_1 e^{-\lambda_2(t-\tau)} + A_2 e^{-\lambda_3(t-\tau)}$$

For both curves c denotes concentration, A, A_1, A_2, λ_1, λ_2 and λ_3 are parameters, t is the observed time and τ is an additional parameter representing a time lag between the recorded and effective zero times. This latter parameter was constrained to be non-negative.

The fitting was carried out using the non-linear maximum likelihood program MLP(1), using weighted least squares with weights proportional to $1/c^2$ (total radioactivity data) or $1/c$ (unchanged WR 178,460 data) (c = observed concentration). The observation taken at 3 days was omitted from the fitting process for unchanged WR 178,460 because of its censored nature (i.e. below the limit of detection).

Study dates

Date of dosing 14 January 1983

Completion of experimental work 18 February 1983

Location of study records

All raw data generated at HRC during the course of this study, together with a copy of this final report have been lodged in the Huntingdon Research Centre plc, Archives, Huntingdon, England.

RESULTS

Excretion of radioactivity (Table 1)

After oral administration of ^{14}C-WR 178,460.HCl to a beagle dog radioactivity was excreted almost entirely via the faeces. Urinary excretion accounted for only 0.1% dose during seven days after dosing. Excretion in faeces accounted for 72.4% dose in the first 24 hours after dosing, 80.8% during the first 48 hours and for a total of 97.2% after seven days. A further 0.7% dose was recovered in cage washes. Total recovery of radioactivity after seven days was 98.0% dose.

Concentrations≠ of radioactivity in plasma and whole-blood
(Table 2, Figures 1, 2 and 4)

Low levels of radioactivity (0.07 μg/ml) were detected in plasma at 15 minutes after dosing and concentrations increased rapidly to 0.64 μg/ml at 2 hours. A peak in radioactivity concentration of 0.68 μg/ml occurred at 4 hours. Concentrations then declined slightly to 0.58 μg/ml at 12 hours before rising to a second peak of 0.73 μg/ml at 30 hours (Figure 1). From 48 hours concentrations appeared to decline biphasically (Figure 2) falling to a level of 0.02 μg/ml after 21 days. The data were fitted to the following triple exponential equation:

$$c = -1270.0e^{-0.622t} + 1215.2e^{-0.0132t} + 54.8e^{-0.00078t}$$

With a time lag (τ) of 0.107 hours half-lives derived from the three exponential coefficients were (standard errors in parentheses) for the absorption phase 1.12 hours (0.25), for the initial elimination phase 52.6 hours (9.5) and for the terminal elimination phase 884 hours (3630) this latter value being determined very imprecisely. Fitted values and residuals are given in Appendix 7. It can be seen that the fitted equation departs from the actual data particularly in the region of the second peak in concentrations.

≠ Where concentrations are reported as μg/ml, radioactivity was assumed to be associated entirely with WR 178,460 free base or with compounds of the same molecular weight

Whole-blood concentrations of radioactivity were slightly lower than those in plasma until 5 hours after dosing (except for 15 minutes where experimental errors were large) and were then higher than in plasma from 5 to 24 hours. A maximum whole-blood : plasma ratio of 1.15 : 1 was measured at 12 hours after dosing. The peak whole-blood concentration occurred at 24 hours (0.72 μg/ml) after which concentrations declined more rapidly than in plasma to be below the limit of accurate measurement (<0.06 μg/ml) after 8 days. Whole-blood : plasma ratios were below 0.8 at 3 days and later times.

Extraction of radioactivity from faeces (Table 3)

The proportion of faecal radioactivity removed by three sequential extracts decreased from 75% for the 0-24 hour sample to 57% and 55% for the 24-48 and 48-72 hour samples respectively. In all three samples a greater proportion of the radioactivity was removed by the second extraction (using methanol : diethylamine, 9 : 1, v/v) than by the first (using methanol alone). Significant amounts of radioactivity were also removed by the third extraction.

Hplc of faecal extracts (Table 4, Appendix 3)

Hplc (reverse phase conditions) resolved the radioactivity in faeces extracts (up to 72 hours after dosing) into three major zones of elution. In the 0-24 hour samples the most important of these eluted with the same retention time (fractions 12-13) as authentic WR 178,460.HCl and accounted for 81.6% and 89.1% of the radioactivity in the first and combined second and third extracts respectively and for a total of 47% dose overall. The next most important zone of elution occurred soon after the solvent front (fractions 3-6) and may have contained more than one metabolite as, resolution in this area would not be expected to be as good as at longer retention times. This zone accounted for 5.4% dose in the total 0-24 hour extracts. A less important zone of elution (fractions 9-10) accounted for 1.3% dose.

Most of the radioactivity (74% or more) in extracts of faeces from 24-48 hours and 48-72 hours eluted in fractions 3-6 and this zone accounted for 3.6% and 3.4% dose respectively during these two time periods. The proportion of extracted radioactivity co-eluting with WR 178,460 decreased with time and amounted to only 0.5% dose in the 24-48 hour extracts and 0.1-0.2% dose in the 48-72 hour extracts.

Hplc of plasma extracts (Tables 5 and 6, Figures 3 and 5, Appendix 4)

The results showed that the proportion of plasma radioactivity which co-eluted with authentic WR 178,460 remained relatively constant (range 50.9-61.0%) in plasma sampled from 1 to 12 hours after dosing. This proportion then declined to 32.2% at 24 hours and to below the limit of accurate measurement (<8.3%) at 72 hours. Calculated concentrations of WR 178,460 free base increased from 0.15 μg/ml at 1 hour to a peak value of 0.40 μg/ml at 4 and 5 hours and then declined to 0.08 μg/ml at 48 hours. The data were fitted to a double exponential equation as follows:

$$c = -0.4478e^{-1.5304t} + 0.4478e^{-0.03316t} \quad (R^2 = 0.9887)$$

with a time lag (τ) of 0.724 hour and associated half-lives for absorption and elimination phases of 0.45 (s.e. 0.11) and 20.9 (s.e. 1.2) hours respectively. The time lag indicated by this fit was substantially greater than that indicated by the radioactivity data (0.107 hours) and so a second fit (Figure 5) was obtained using the latter value for the time lag:

$$c = -0.4981e^{-0.6002t} + 0.4981e^{-0.03602t} \quad (R^2 = 0.9519)$$

which gave half-lives of 1.15 (s.e. 0.20) and 19.2 (s.e. 2.3) hours for the absorption and elimination phases. Fitted data and residuals for both equations are shown in Appendix 8 from which it can be seen that the fit is substantially better with the longer time lag.

Radioactivity in plasma which did not co-elute with WR 178,460 was associated with two other zones of elution. The major of these occurred soon after the solvent front (fractions 3-5, Table 6) and accounted for a relatively constant proportion of plasma radioactivity (34-40%) in samples from 2 to 12 hours and for an increasing proportion at later times up to 100% at 72 hours. A minor zone of elution, associated with a single fraction in each sample between fractions 8 and 10 accounted for between 5 and 10% of radioactivity in most plasma samples. Overall the elution times of radioactive fractions in plasma extracts seemed to correspond closely with those observed for extracts of faeces.

Mass spectrometry (Appendix 1)

Mass spectrometry was used to confirm the chemical identity of ^{14}C-WR 178,460.HCl. Mass spectra of ^{14}C-WR 178,460.HCl and authentic non-radioactive WR 178,460.HCl were essentially identical, with the exception of altered peak intensity ratios due to the presence of carbon-14.

DISCUSSION

The extent of absorption of ^{14}C-WR 178,460.HCl, after oral administration in suspension to a beagle dog, remains questionable as radioactivity was eliminated almost entirely *via* the faeces. During 7 days after dosing only 0.12% dose was excreted in the urine. A large proportion (72.4%) of the dose was eliminated *via* the faeces within 24 hours and a considerable part of this material was probably associated with unabsorbed compound. Solvent extraction removed 75% of the radioactivity in the first 24 hour faeces sample and about 87% of this extracted radioactivity, equivalent to 47% dose, was shown to co-chromatograph with unchanged WR 178,460.HCl. The figure of 47% dose represents a minimum value for unchanged compound as additional extractions would certainly have removed further faecal radioactivity. A minor proportion of the 0-24 hour faecal radioactivity and a major proportion from samples collected between 24 and 72 hours was associated with metabolites which were more mobile under reverse phase chromatographic conditions than WR 178,460.HCl and therefore probably more polar. These metabolites accounted for 16% dose in all faecal extracts up to 72 hours. It is likely that these metabolites represented absorbed material, (and hence a minimum value for absorption of the dose) which had been excreted in the bile, especially since the molecular weight of WR 178,460 is greater than the threshold considered necessary for active biliary excretion in the dog.

The profile of the plasma radioactivity concentration-time curve was further suggestive of the occurrence of biliary excretion of radioactivity and the 30 hour peak in concentrations possibly resulted from re-absorption of material already excreted by that route. Peak concentrations of unchanged WR 178,460 in plasma occurred at 4 to 5 hours after dosing and up to 12 hours WR 178,460 represented a relatively constant proportion of plasma radioactivity. The decline in WR 178,460 concentrations appeared to follow first order kinetics with a half-life of 19 to 21 hours and by 48 hours concentrations had fallen to a low level. The decline in total radioactivity concentrations in plasma did not begin until after 48 hours and therefore reflects the elimination of metabolites of ^{14}C-178,460 rather than unchanged compound. The decline appeared to be biphasic in nature with the initial more rapid phase lasting until about 8 days after dosing and having a half-life of 52.6 hours. The half-life of the terminal phase was much longer than the period for which measurements were available and so could not be determined with any degree of precision.

The results, discussed above from the study with WR 178,460.HCl are closely comparable to results obtained in a pilot dog study with the parent compound WR 171,669.HCl (Study Report No. 1, Hawkins et al. 1983). The patterns and ratios of excretion of the two compounds were very similar although excretion of radioactivity in the first 24-hour faeces was greater after administration of WR 178,460.HCl. Profiles of plasma radioactivity concentrations for the two compounds were also similar, and although a complete profile of unchanged WR 171,669 concentrations in plasma was not obtained, a limited range of results indicated a similar trend to that observed in the present study with WR 178,460. The metabolism of the two compounds may also follow a similar pathway as in both studies the principal metabolic products behaved in a similar manner during chromatography. This possibility will be investigated during the definitive study with ^{14}C-WR 171,669.HCl in dogs.

REFERENCE

Hawkins D.R., Kirkpatrick D., Jackson, A.J.S.

Interim Report No. 1, HRC Confidential Report No. WRI 1/8335.

TABLE 1

Excretion of radioactivity after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog at a dose level of 21 mg/kg

Results are expressed as % dose

Time (hours)	Radioactivity excreted			Cumulative excretion			
	Urine	Faeces	Cage wash	Urine	Faeces	Cage wash	Total
0- 24	0.05	72.4	0.20	0.05	72.4	0.20	72.6
24- 48	0.02	8.4	0.12	0.07	80.8	0.32	81.2
48- 72	0.02	7.2	0.12	0.09	88.0	0.44	88.5
72- 96	0.009	4.9	0.09	0.10	92.9	0.53	93.6
96-120	0.007	2.1	0.11	0.11	95.0	0.64	95.8
120-144	0.006	1.2	0.03	0.12	96.2	0.67	97.0
144-168	0.004	1.0	0.01	0.12	97.2	0.68	98.0

TABLE 2

Concentrations of radioactivity in plasma and whole-blood after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog at a dose level of 21 mg/kg

Time	µg equivalents WR 178,460*/ml		% dose/litre	
	Plasma	Whole-blood/	Plasma	Whole-blood/
15 min	0.07	0.07	0.03	0.03
30 min	0.20	0.18	0.08	0.08
1 hr	0.30	0.29	0.12	0.12
2 hrs	0.64	0.52	0.26	0.21
3 hrs	0.66	0.62	0.27	0.25
4 hrs	0.68	0.66	0.28	0.27
5 hrs	0.66	0.69	0.27	0.28
6 hrs	0.64	0.69	0.26	0.28
7 hrs	0.61	0.70	0.25	0.29
12 hrs	0.58	0.67	0.24	0.27
24 hrs	0.71	0.72	0.29	0.29
30 hrs	0.73	0.70	0.30	0.29
2 days	0.66	0.57	0.27	0.23
3 days	0.48	0.31	0.20	0.13
4 days	0.30	0.24	0.12	0.10
5 days	0.20	0.14	0.08	0.06
6 days	0.14	0.08	0.06	0.03
7 days	0.10	0.07	0.04	0.03
8 days	0.09	< 0.06	0.04	< 0.03
10 days	0.05	< 0.06	0.02	< 0.03
12 days	0.05	< 0.06	0.02	< 0.03
14 days	0.04	< 0.06	0.02	< 0.03
18 days	0.03	< 0.06	0.01	< 0.03
21 days	0.02	< 0.06	0.009	< 0.03

* Calculated as free base

/ A value of 1.052, for the specific gravity of dog blood, was used in calculation of these results from data in Appendix 6

TABLE 3

Extraction≠ of radioactivity from faeces samples up to 72 hours after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog at a dose level of 21 mg/kg

Results are expressed as % faecal radioactivity

Time period (hours)	Extract 1	Extract 2	Extract 3	Total*
0 - 24	24	31	21	75 (54.6)
24 - 48	19	23	14	57 (4.8)
48 - 72	9	30	17	55 (4.0)

* Total is calculated from individual results to one decimal place and then rounded to nearest whole number
Figures in brackets are % dose

≠ Extract 1; methanol, Extract 2, 3; methanol : diethylamine 9 : 1, v/v

TABLE 4

Radioactivity in one minute≠ fractions of hplc eluate after injection of extracts of faeces collected up to 72 hours after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog at a dose level of 21 mg/kg

Time interval (hours)	Fraction numbers (inclusive)	% eluted radioactivity		Total % dose (all 3 extracts)
		Extract 1	Extract 2 + 3	
0 - 24	3 - 6	14.4	7.7	5.4
	9 - 10	3.9	1.6	1.3
	12 - 13*	81.6	89.1+	47.0
	Others	-	1.6	0.9
24 - 48	3 - 6	74.6	76.9	3.6
	9 - 10	12.0	6.7	0.4
	12 - 13*	13.4	9.8/	0.5
	Others	-	6.6	0.2
48 - 72	3 - 6	83.1	87.2	3.4
	9 - 10	16.9	5.5	0.3
	12 - 13*	<5.0	4.2/	0.1 - 0.2
	Others	-	3.1	0.1

* Corresponds to WR 178,460
\+ Includes 1.5% from fractions 14 and 15
/ Fractions 11, 12 (not 13)
≠ For results in detail see Appendix 3

TABLE 5

Concentrations of WR 178,460* free base in plasma after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog at a dose level of 21 mg/kg

Time (hours)	Concentration (µg/ml)	Proportion of total plasma radioactivity (%)
1	0.15	50.9
2	0.37	57.6
3	0.39	59.5
4	0.40	59.5
5	0.40	61.0
6	0.37	57.1
7	0.34	55.0
12	0.32	54.6
24	0.23	32.2
30	0.18	24.7
48	0.08	12.9
72	<0.04	<8.3

* Refers to radioactivity eluting from an hplc column with the same retention time as WR 178,460

Results are not corrected for recovery

TABLE 6

Radioactivity in one minute fractions≠ of hplc eluate after injection of extracts of plasma collected up to 72 hours after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog at a dose level of 21 mg/kg

Results are expressed as % eluted radioactivity

Time (hours)	Fractions 3 - 5	Fractions 8 - 10	Fractions 11 - 13*
1	49.1	<9.6	50.9
2	36.2	6.2	57.6
3	35.2	5.3	59.6
4	34.3	6.2	59.5
5	34.0	5.0	61.0
6	37.8	5.1	57.1
7	39.5	5.6	55.0
12	35.9	9.5	54.6≠
24	59.8	8.0	32.2
30	65.5	9.8	24.7.
48	78.9	8.2	12.9
72	100	<8.3	<8.3

≠ For results in detail see Appendix 4
≠ Fractions 13 - 14
* Corresponds to WR 178,460

FIGURE 1

Concentrations of radioactivity in plasma and whole-blood up to 48 hours after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog

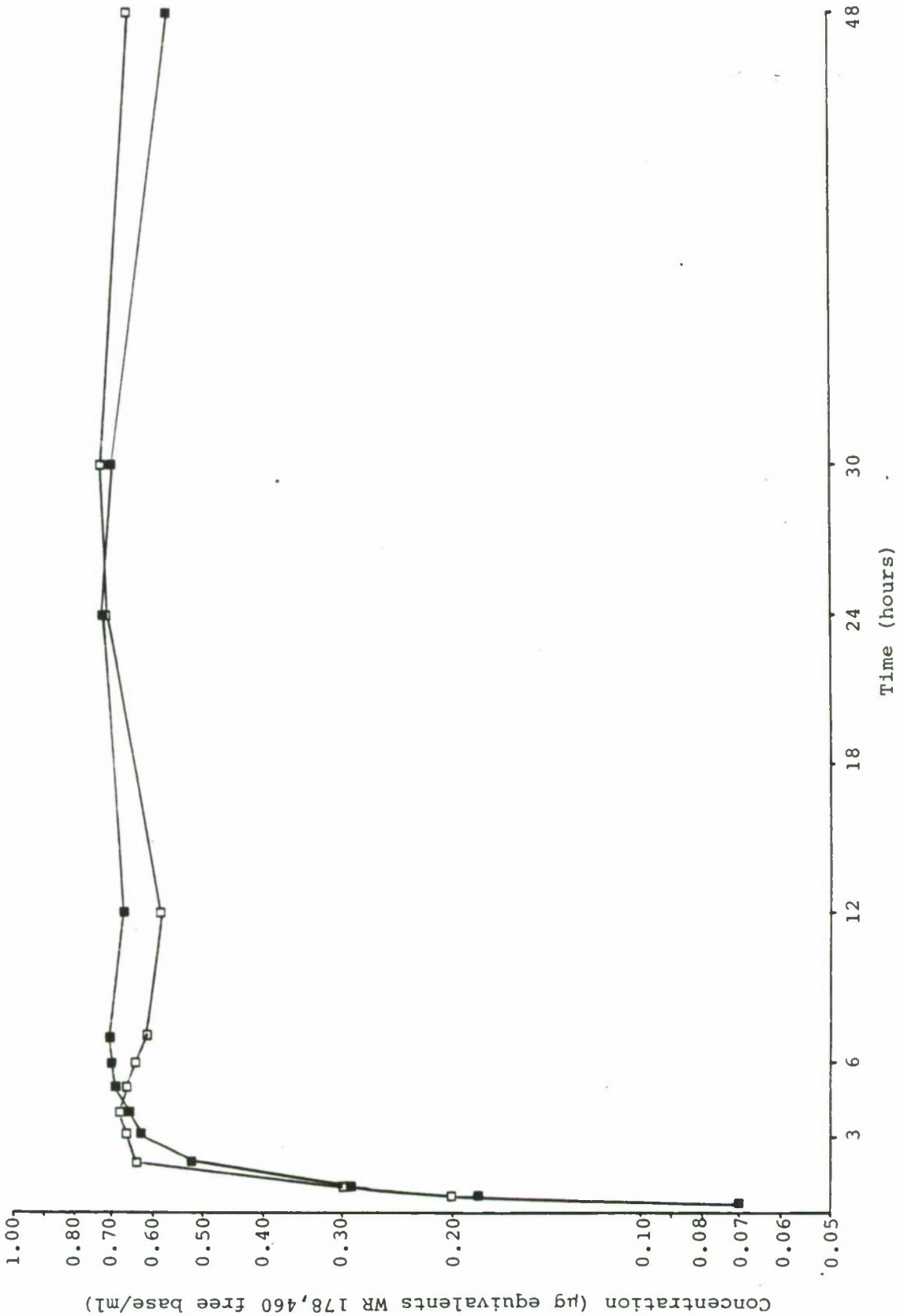


FIGURE 2

Concentrations of radioactivity in plasma and whole-blood up to 21 days after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog

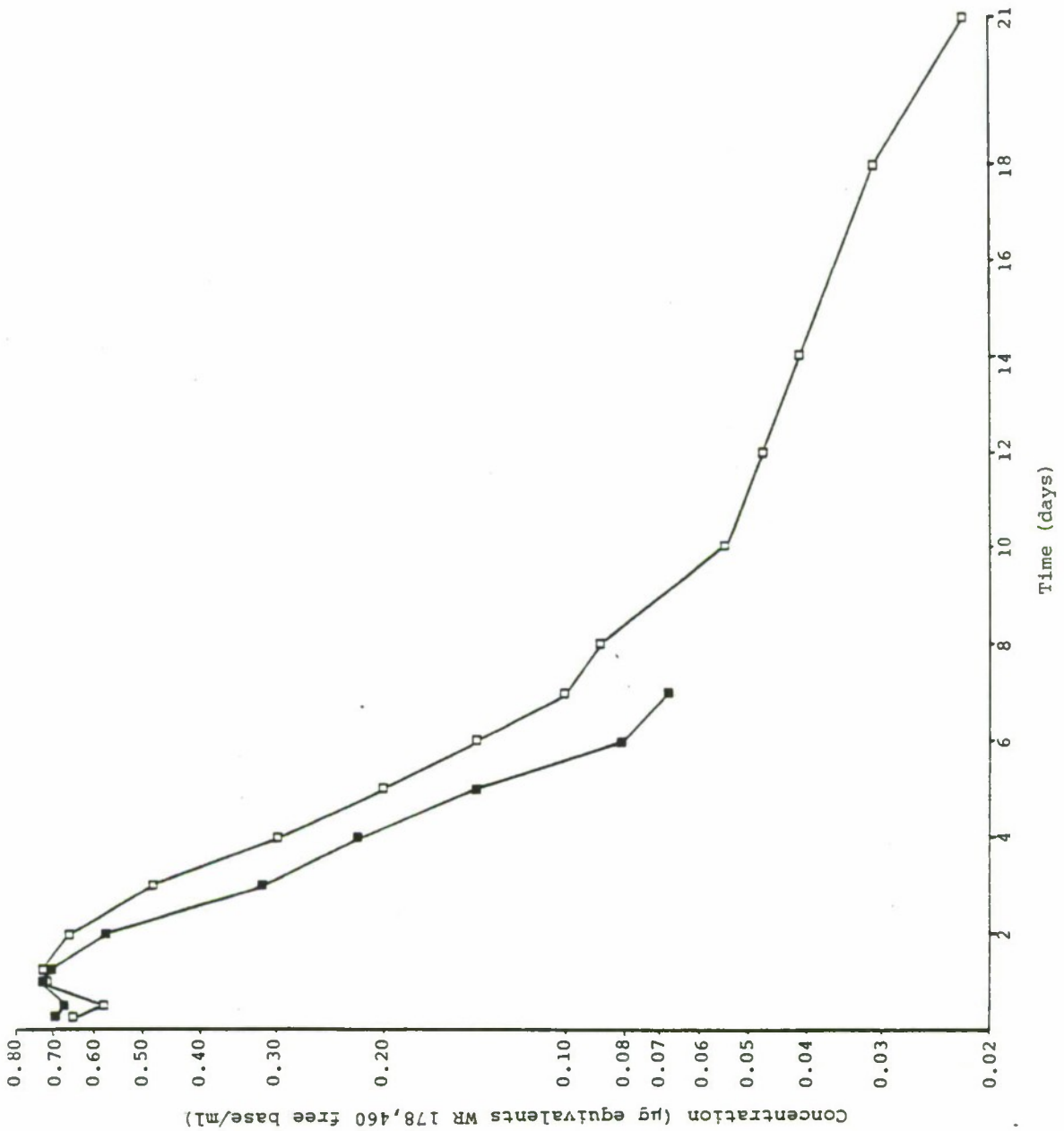


FIGURE 3

Concentrations of radioactivity and of WR 178,460 in plasma after administration of ^{14}C-WR 178,460.HCl to a beagle dog

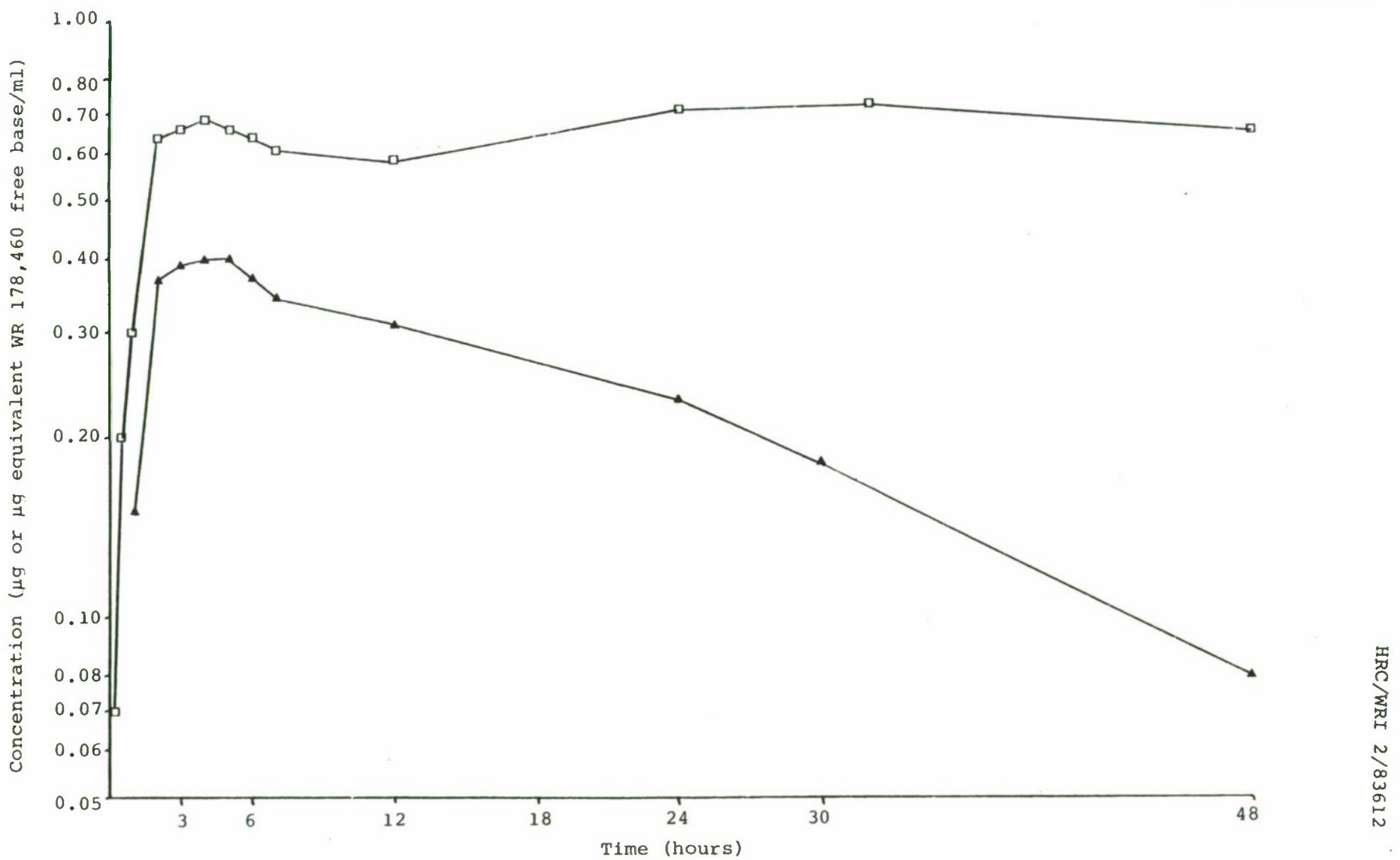


FIGURE 4

Observed points (▲) and fitted curve for concentrations of total radioactivity in plasma after oral administration of ^{14}C-WR 178,460.HCl to a beagle dog

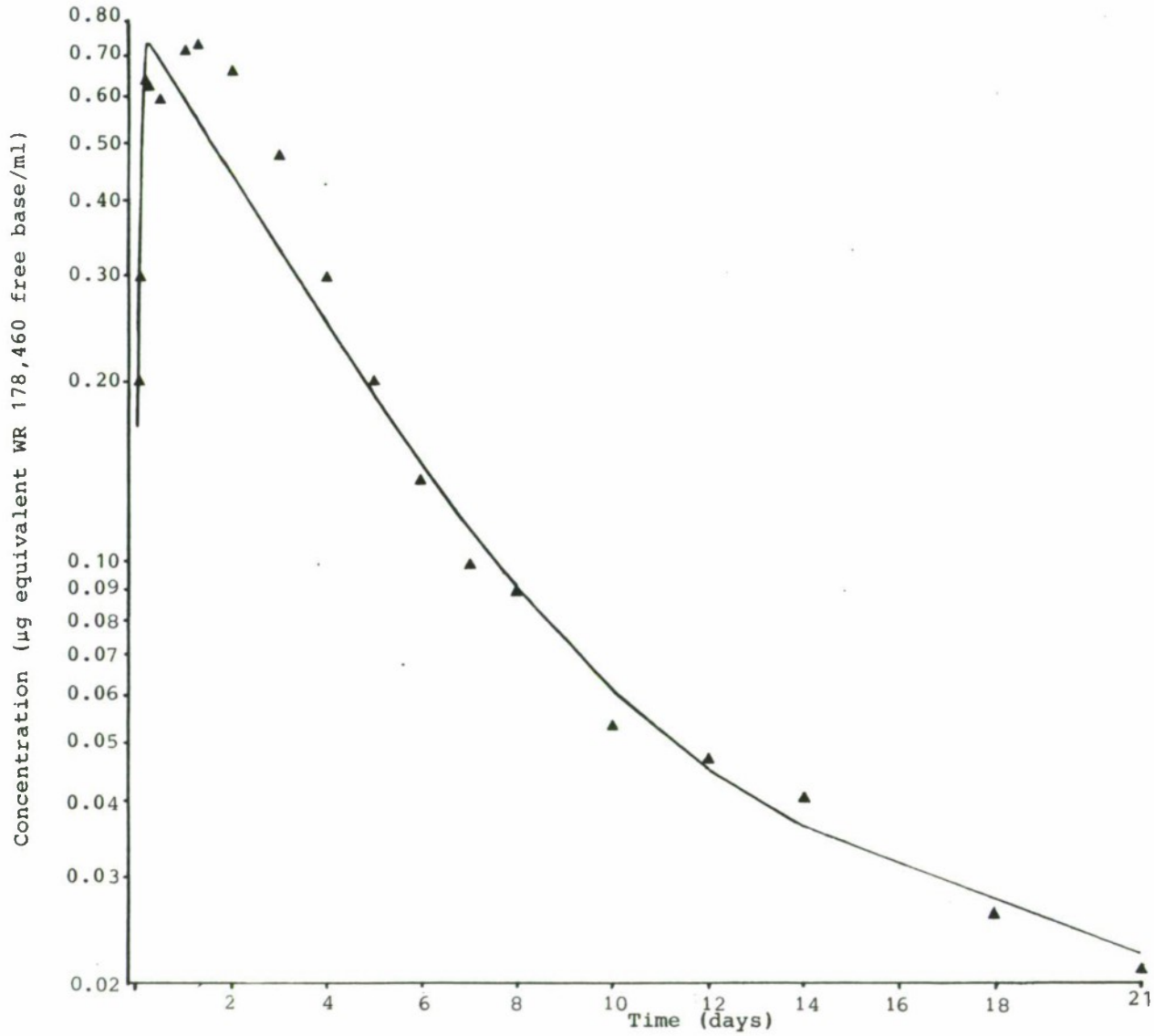
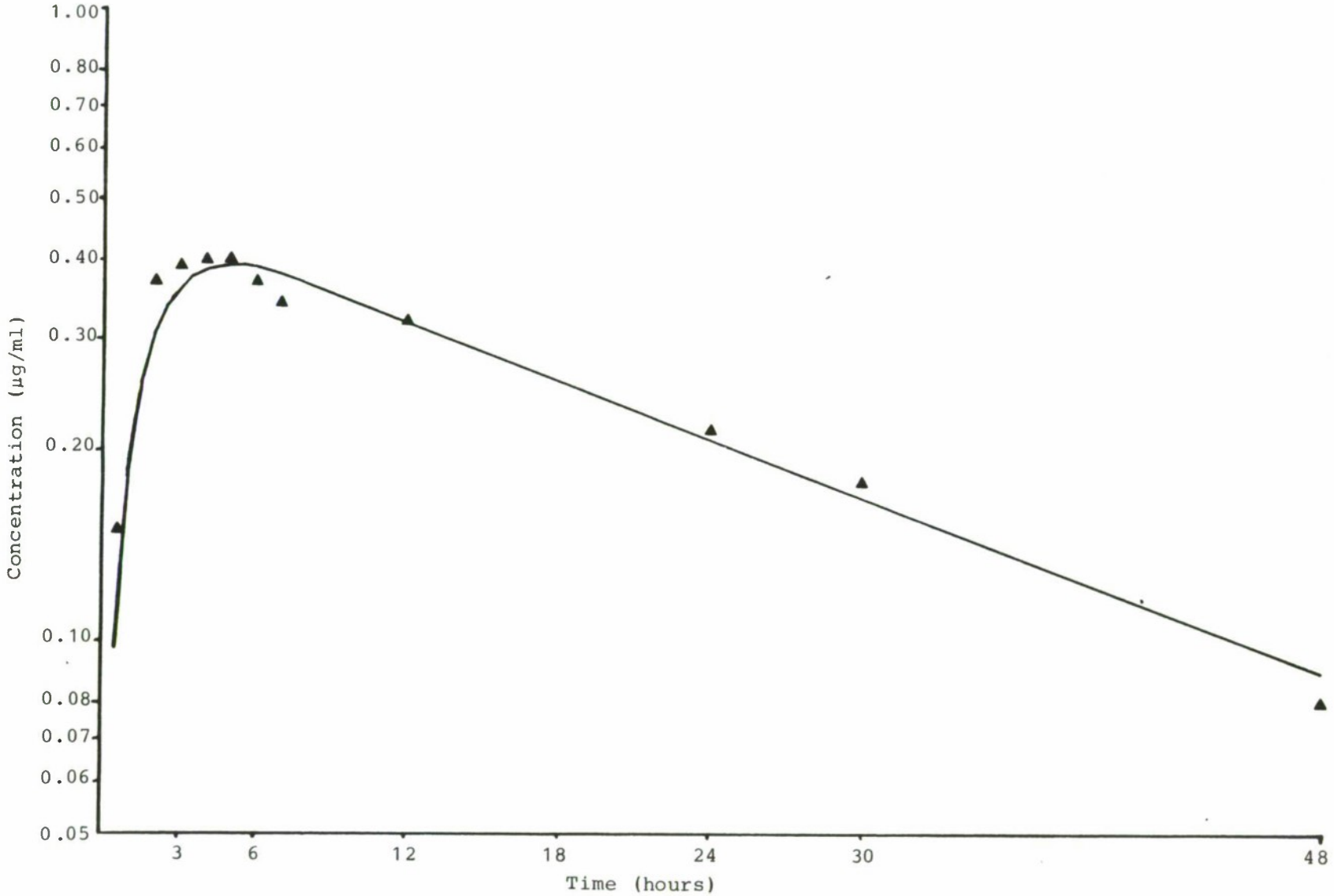


FIGURE 5

Observed points (▲) and fitted curve for concentrations of WR 178,460. in plasma after administration of ^{14}C-WR 178,460.HCl to a beagle dog (time lag - 0.107 hours)

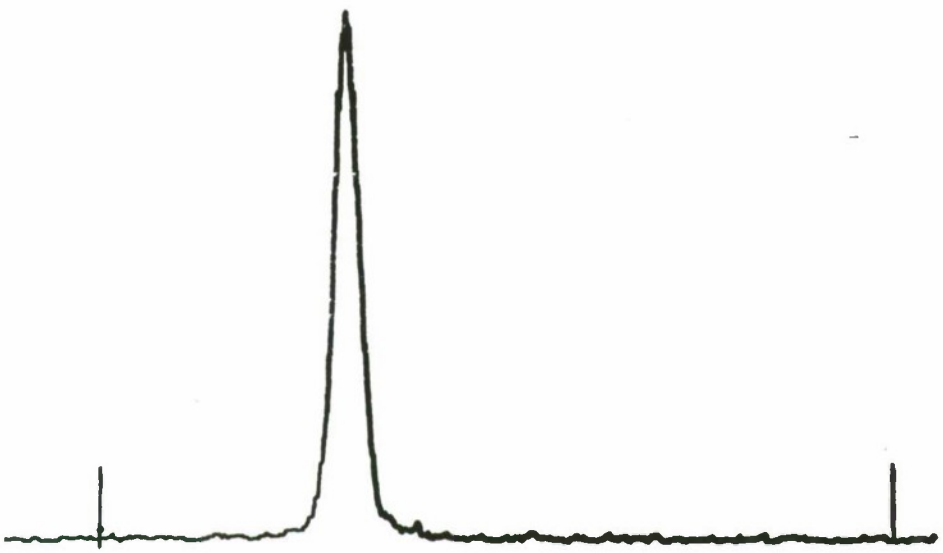


Specification of ^{14}C-WR 178,460.HCl
(as supplied by Research Triangle Institute)

Lot No.	3793-91
Stated specific activity	16.0 Ci/mol (33.4 mCi/g)
Mass spectrum (recorded at HRC)	Essentially identical to spectrum of authentic non-radioactive WR 178,460.HCl except for altered peak intensity ratios due to presence of ^{14}C.
Radiochemical purity (measured at HRC)	Tetrahydrofuran : acetic acid (1 : 1, v/v) 97.7%. Chloroform : methanol : 35% aqueous ammonia (90 : 10 : 1, v/v) 96.4%. (This value was considered satisfactory as Research Triangle report some decomposition of WR 178,460.HCl in solvent systems of this type).

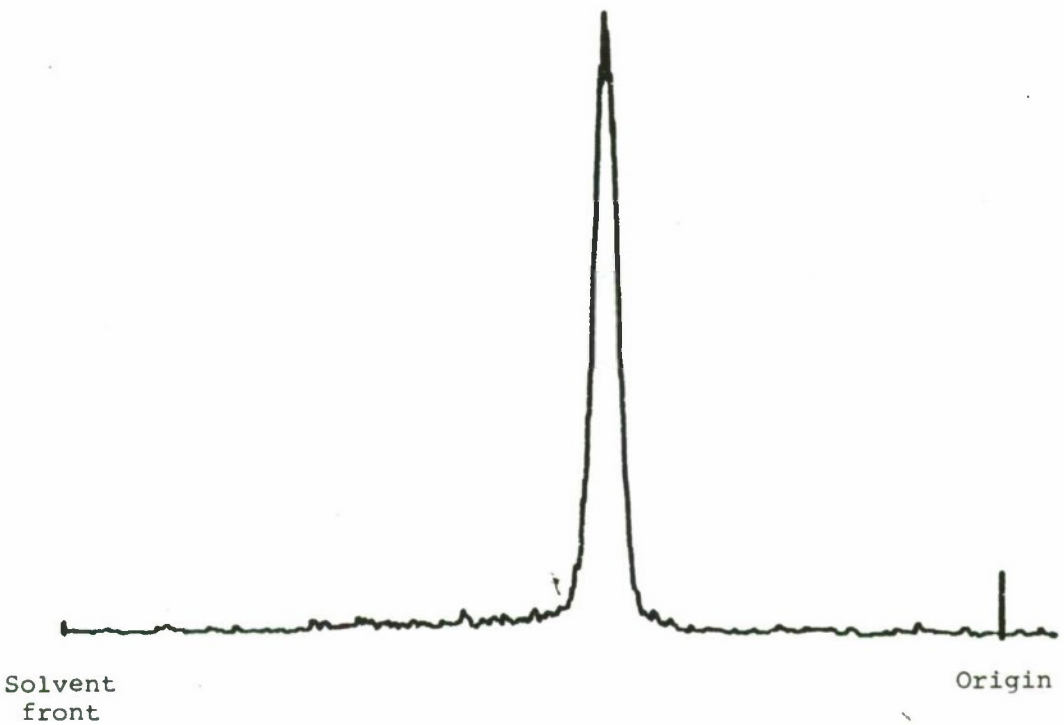
(continued)

Thin-layer radiochromatogram of ^{14}C-WR 178,460.HCl
(Solvent system and tetrahydrofuran : acetic acid, 1 : 1, v/v)



(continued)

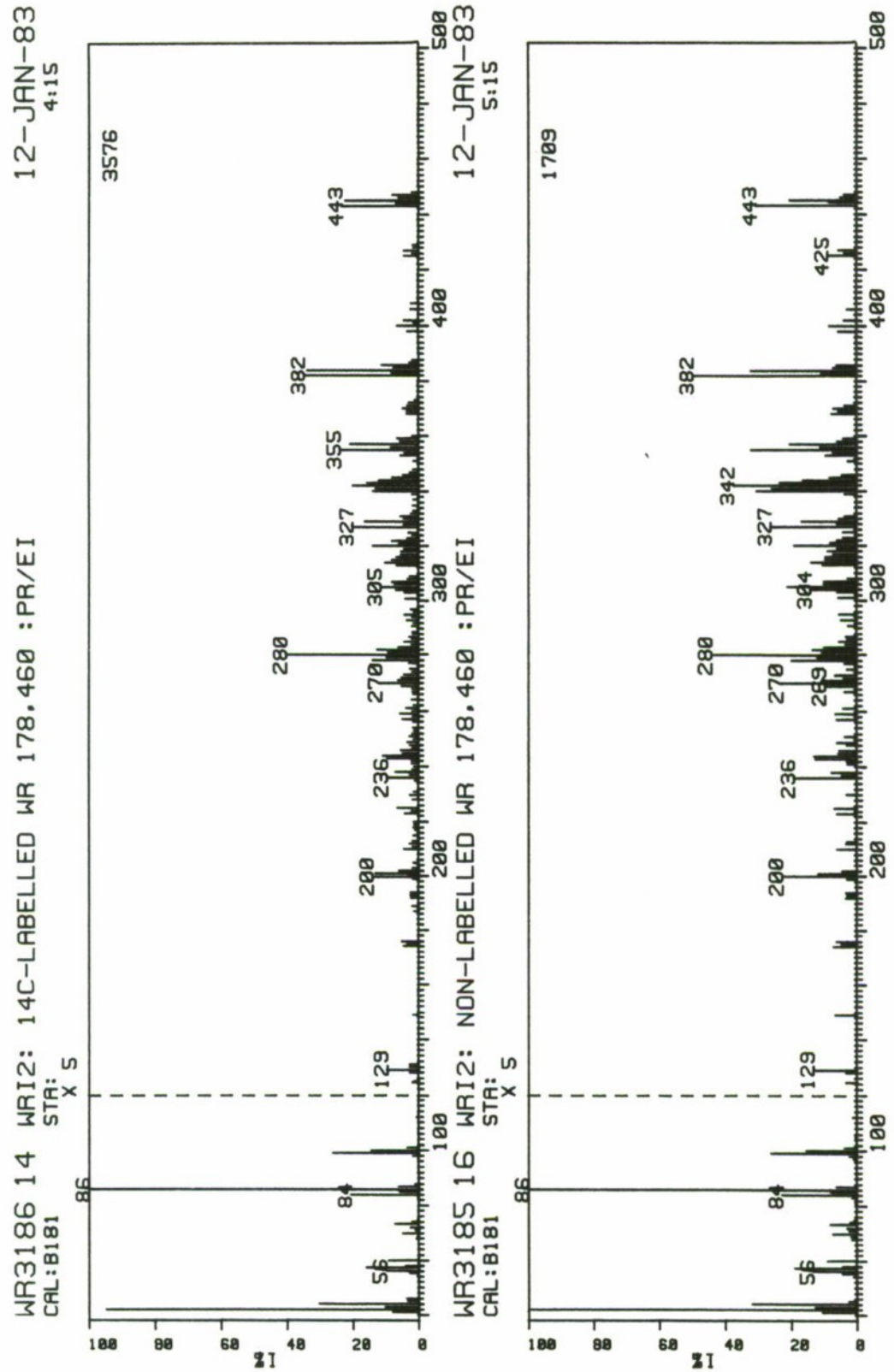
Thin-layer radiochromatogram scan of ^{14}C-WR 178,460.HCl
(Solvent system : chloroform : methanol : 35% aqueous ammonia,
90 : 10 : 1, v/v)



APPENDIX 1

(continued)

Mass spectra of ^{14}C-WR 178,460.HCl and non-radioactive WR 178,460.HCl



APPENDIX 2

Preparation of dose

Weight ^{14}C-WR 178,460.HCl	6.01 mg
Weight non-radioactive WR 178,460.HCl	268.7 mg
Volume of dose suspension	14 ml
Total radioactivity in dose	399,026,000 dpm*
Total radioactivity in dose washings	13,160,000 dpm
Net dose (radioactivity)	385,866,000 dpm
Net dose (weight)	265 mg
Specific activity of administered ^{14}C-WR 178,460.HCl	1456 dpm/µg (0.66 µCi/mg)
Calculated specific activity of ^{14}C-WR 178,460 free base	1576 dpm/µg

* After removal of 5 x 5 µl aliquots from dose dissolved in 10 ml of methanol

APPENDIX 3

Hplc of faecal extracts

(Results expressed as dpm)

TABLE 1

ELUTION FROM HPLC COLUMN OF FAECES EXTRACT 1 DOG 1,0-24 HOURS

BACKGROUND = 28;LIMIT OF DETECTION = 28.0 .84 .841%
TOTAL DPM ELUTED = 3330

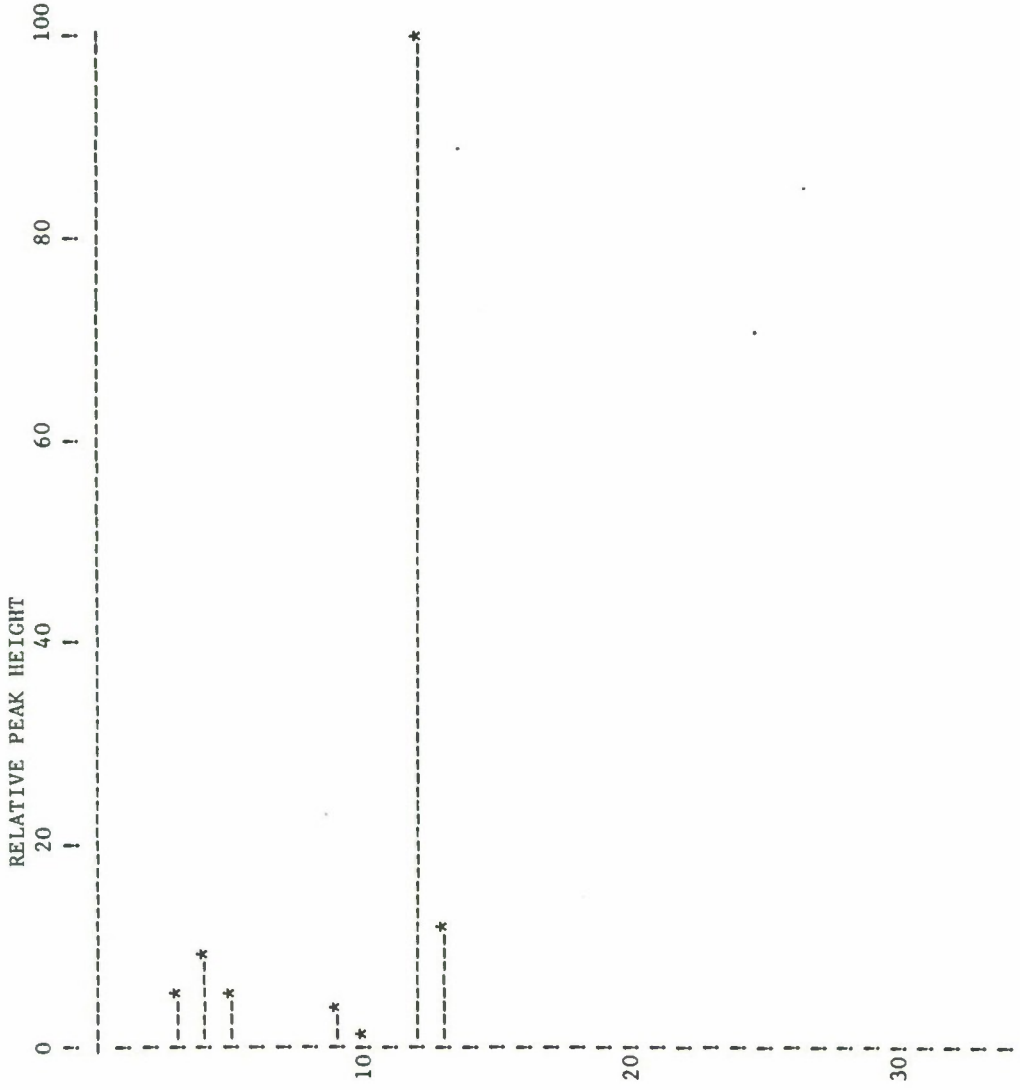
FRACTION(MINS)	GROSS	NET	%	%
1	22.	0.	.0	.00
2	46.	0.	.0	.00
3	165.	137.	4.1	4.11
4	257.	229.	6.9	6.88
5	143.	115.	3.5	3.45
6	49.	0.	.0	.00
7	40.	0.	.0	.00
8	54.	0.	.0	.00
9	117.	89.	2.7	2.67
10	70.	42.	1.3	1.26
11	53.	0.	.0	.00
12	2462.	2434.	73.1	73.09
13	312.	284.	8.5	8.53
14	54.	0.	.0	.00
15	41.	0.	.0	.00
16	32.	0.	.0	.00
17	34.	0.	.0	.00
18	33.	0.	.0	.00
19	29.	0.	.0	.00
20	26.	0.	.0	.00
21	29.	0.	.0	.00
22	33.	0.	.0	.00
23	25.	0.	.0	.00
24	31.	0.	.0	.00
25	25.	0.	.0	.00
26	24.	0.	.0	.00
27	26.	0.	.0	.00
28	23.	0.	.0	.00
29	24.	0.	.0	.00
30	28.	0.	.0	.00
31	27.	0.	.0	.00
32	24.	0.	.0	.00
33	23.	0.	.0	.00
34	20.	0.	.0	.00

APPENDIX 3

(continued)

FIGURE 1

Elution from hplc column of faeces extract 1 dog 1,0-24 hours



APPENDIX 3

(continued)

TABLE 2

ELUTION FROM HPLC COLUMN OF FAECES EXTRACTS 2 + 3 DOG 1, 0-24 HOURS

BACKGROUND = 28;LIMIT OF DETECTION = 28.0 .25 .245%
TOTAL DPM ELUTED = 11421

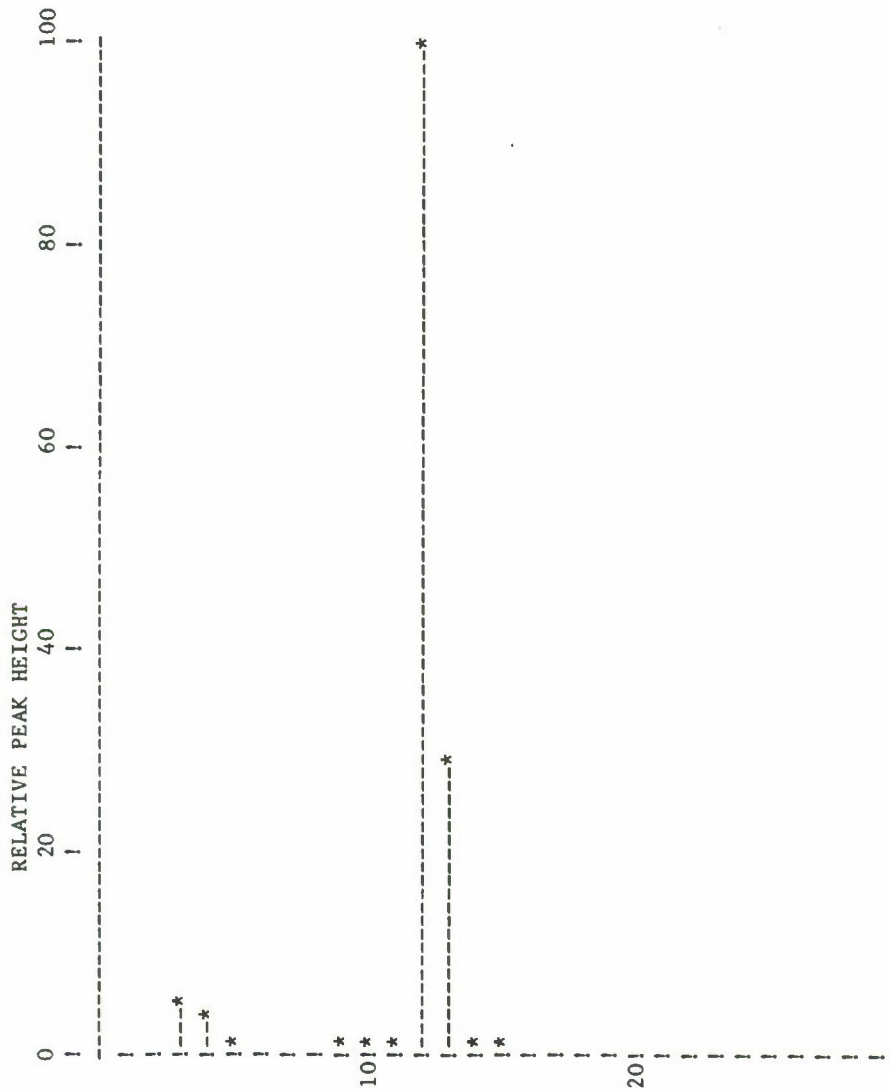
FRACTION(MINS)	GROSS	NET	%	%
1	26.	0.	.0	.00
2	72.	44.	.4	.39
3	487.	459.	4.0	4.02
4	293.	265.	2.3	2.32
5	143.	115.	1.0	1.01
6	66.	38.	.3	.33
7	57.	29.	.3	.25
8	75.	47.	.4	.41
9	153.	125.	1.1	1.09
10	90.	62.	.5	.54
11	94.	66.	.6	.58
12	7783.	7755.	67.9	67.90
13	2274.	2246.	19.7	19.67
14	139.	111.	1.0	.97
15	87.	59.	.5	.52
16	55.	0.	.0	.00
17	34.	0.	.0	.00
18	38.	0.	.0	.00
19	33.	0.	.0	.00
20	35.	0.	.0	.00
21	29.	0.	.0	.00
22	31.	0.	.0	.00
23	25.	0.	.0	.00
24	32.	0.	.0	.00
25	33.	0.	.0	.00
26	32.	0.	.0	.00
27	26.	0.	.0	.00
28	29.	0.	.0	.00
29	28.	0.	.0	.00

APPENDIX 3

(continued)

FIGURE 2

Elution from hplc column of faeces extracts 2 + 3 dog 1, 0-24 hours



APPENDIX 3

(continued)

TABLE 3

ELUTION FROM HPLC COLUMN OF FAECES EXTRACT 1 DOG 1,24-48 HOURS

BACKGROUND = 28;LIMIT OF DETECTION = 28.0 1.55 1.546%
TOTAL DPM ELUTED = 1811

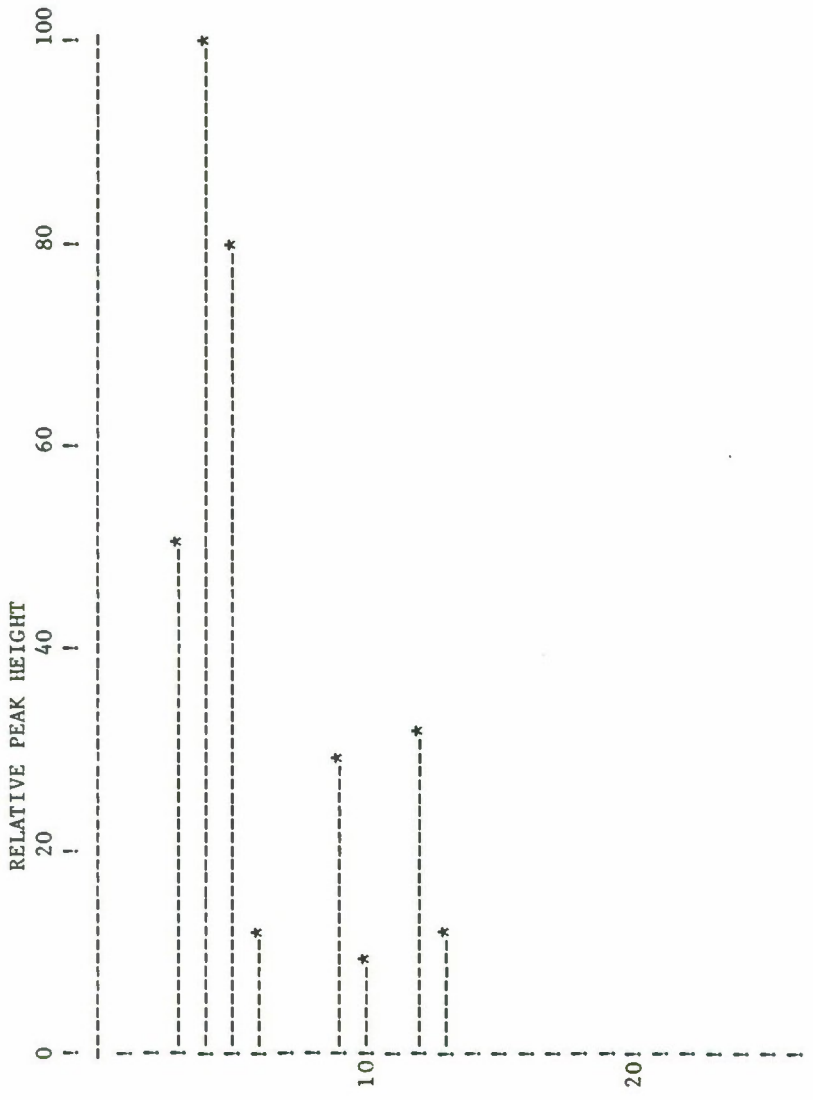
FRACTION(MINS)	GROSS	NET	%	%
1	34.	0.	.0	.00
2	42.	0.	.0	.00
3	312.	284.	15.7	15.68
4	583.	555.	30.6	30.65
5	474.	446.	24.6	24.63
6	94.	66.	3.6	3.64
7	54.	0.	.0	.00
8	51.	0.	.0	.00
9	194.	166.	9.2	9.17
10	80.	52.	2.9	2.87
11	35.	0.	.0	.00
12	206.	178.	9.8	9.83
13	92.	64.	3.5	3.53
14	34.	0.	.0	.00
15	29.	0.	.0	.00
16	32.	0.	.0	.00
17	30.	0.	.0	.00
18	25.	0.	.0	.00
19	28.	0.	.0	.00
20	26.	0.	.0	.00
21	24.	0.	.0	.00
22	27.	0.	.0	.00
23	26.	0.	.0	.00
24	25.	0.	.0	.00
25	27.	0.	.0	.00
26	29.	0.	.0	.00

APPENDIX 3

(continued)

FIGURE 3

Elution from hplc column of faeces extract 1 dog 1, 24-48 hours



(continued)

TABLE 4

ELUTION FROM HPLC COLUMN OF FAECES EXTRACTS 2 + 3 DOG 1 ,24-48 HOURS

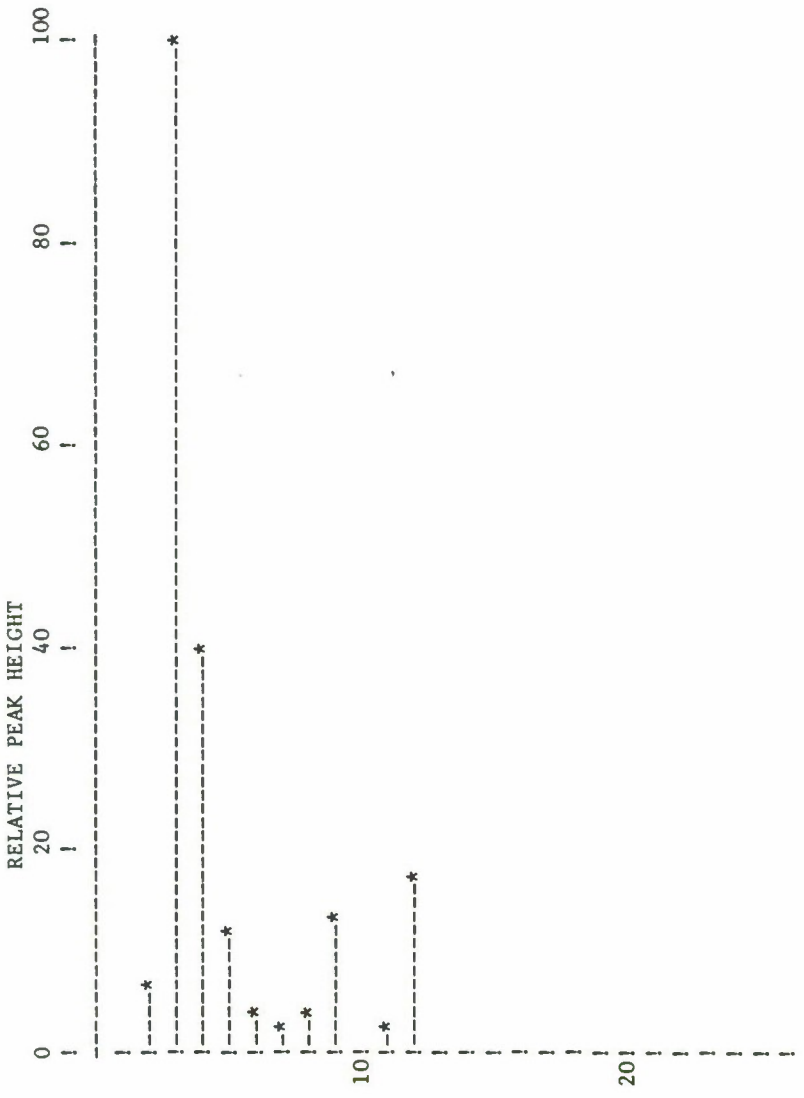
BACKGROUND = 28;LIMIT OF DETECTION = 28.0 .97 .965%
TOTAL DPM ELUTED = 2901

FRACTION(MINS)	GROSS	NET	%	%
1	29.	0.	.0	.00
2	130.	102.	3.5	3.52
3	1462.	1434.	49.4	49.43
4	605.	577.	19.9	19.89
5	199.	171.	5.9	5.89
6	77.	49.	1.7	1.69
7	64.	36.	1.2	1.24
8	81.	53.	1.8	1.83
9	222.	194.	6.7	6.69
10	52.	0.	.0	.00
11	58.	30.	1.0	1.03
12	283.	255.	8.8	8.79
13	50.	0.	.0	.00
14	34.	0.	.0	.00
15	30.	0.	.0	.00
16	30.	0.	.0	.00
17	23.	0.	.0	.00
18	31.	0.	.0	.00
19	30.	0.	.0	.00
20	25.	0.	.0	.00
21	25.	0.	.0	.00
22	29.	0.	.0	.00
23	33.	0.	.0	.00
24	31.	0.	.0	.00
25	26.	0.	.0	.00
26	21.	0.	.0	.00

(continued)

FIGURE 4

Elution from hplc column of faeces extracts 2 + 3 dog 1, 24-48 hours



APPENDIX 3

(continued)

TABLE 5

ELUTION FROM HPLC COLUMN OF FAECES EXTRACT 1 DOG 1,48-72 HOURS

BACKGROUND = 28;LIMIT OF DETECTION = 28.0 3.44 3.444%
TOTAL DPM ELUTED = 813

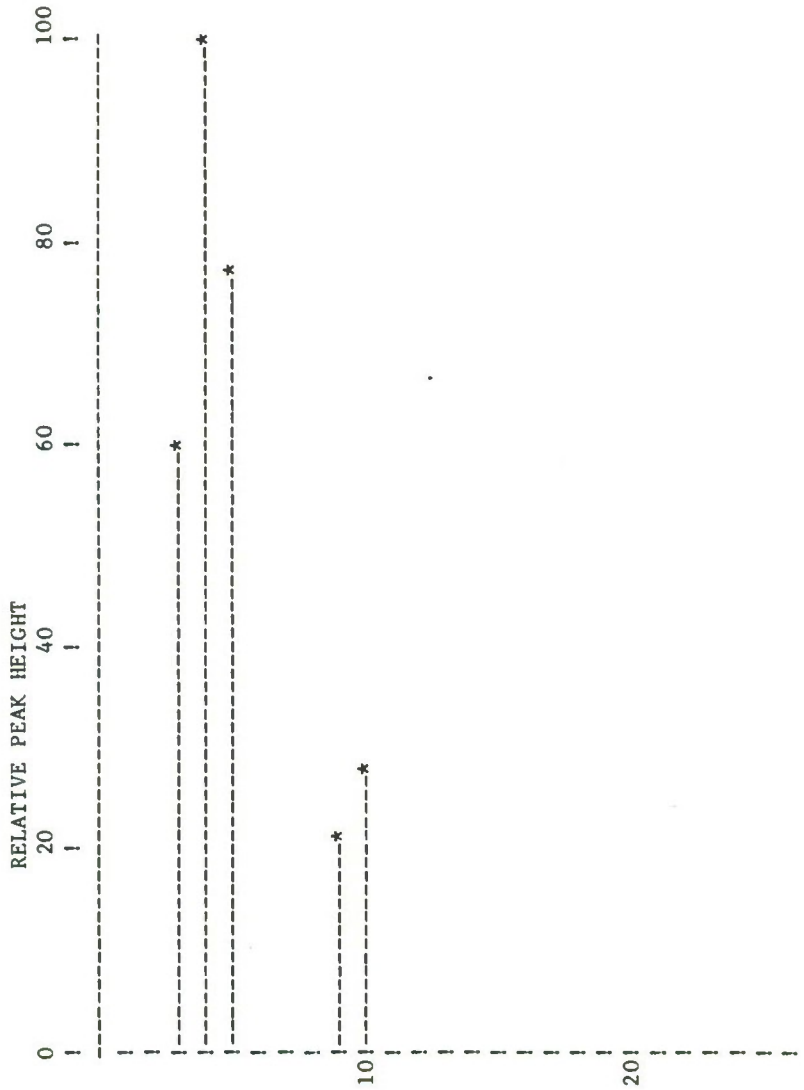
FRACTION(MINS)	GROSS	NET	%	%
1	27.	0.	.0	.00
2	26.	0.	.0	.00
3	199.	171.	21.0	21.03
4	313.	285.	35.1	35.06
5	248.	220.	27.1	27.06
6	44.	0.	.0	.00
7	34.	0.	.0	.00
8	32.	0.	.0	.00
9	87.	59.	7.3	7.26
10	106.	78.	9.6	9.59
11	34.	0.	.0	.00
12	37.	0.	.0	.00
13	46.	0.	.0	.00
14	28.	0.	.0	.00
15	31.	0.	.0	.00
16	25.	0.	.0	.00
17	29.	0.	.0	.00
18	28.	0.	.0	.00
19	26.	0.	.0	.00
20	25.	0.	.0	.00
21	27.	0.	.0	.00
22	27.	0.	.0	.00
23	28.	0.	.0	.00
24	24.	0.	.0	.00
25	28.	0.	.0	.00
26	25.	0.	.0	.00

APPENDIX 3

(continued)

FIGURE 5

Elution from hplc column of faeces extract 1 dog 1, 48-72 hours



APPENDIX 3

(continued)

TABLE 6

ELUTION FROM HPLC COLUMN OF FAECES EXTRACTS 2 + 3 DOG 1,48-72 HOURS

BACKGROUND = 28;LIMIT OF DETECTION = 28.0 .93 .934%
TOTAL DPM ELUTED = 2997

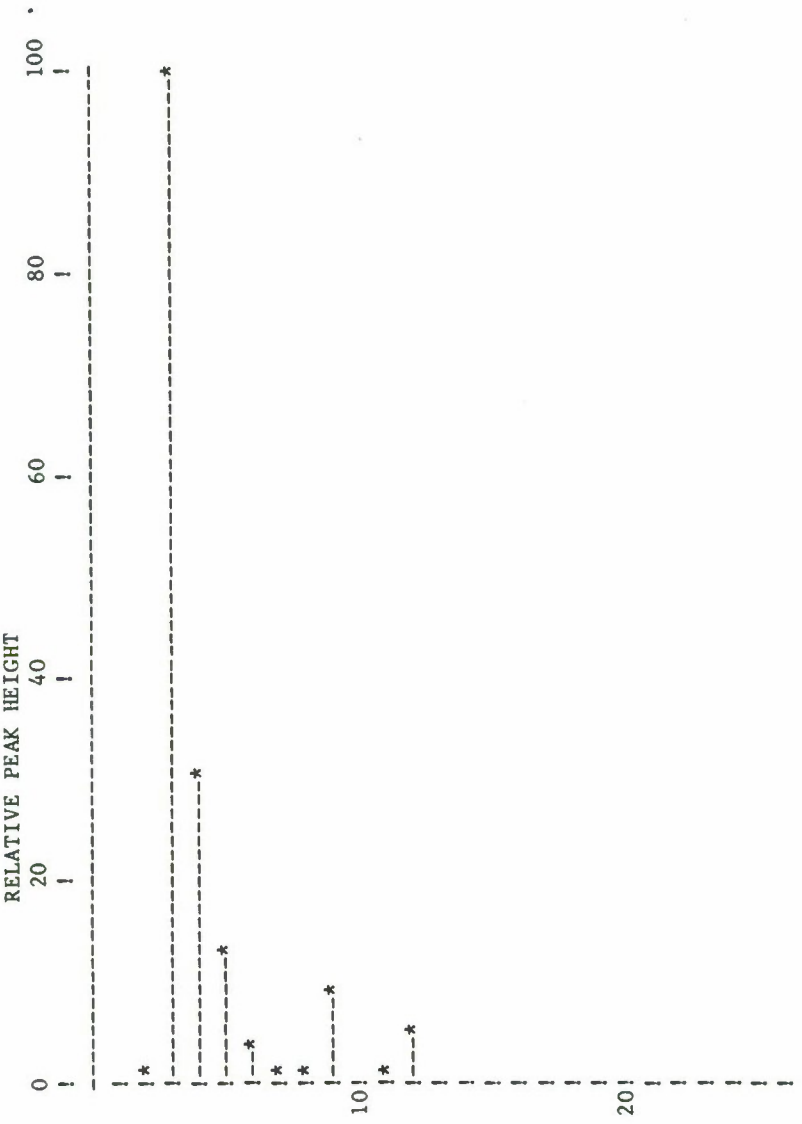
FRACTION(MINS)	GROSS	NET	%	%
1	30.	0.	.0	.00
2	57.	29.	1.0	.97
3	1804.	1776.	59.3	59.26
4	568.	540.	18.0	18.02
5	262.	234.	7.8	7.81
6	92.	64.	2.1	2.14
7	63.	35.	1.2	1.17
8	56.	28.	.9	.93
9	192.	164.	5.5	5.47
10	53.	0.	.0	.00
11	58.	30.	1.0	1.00
12	125.	97.	3.2	3.24
13	53.	0.	.0	.00
14	33.	0.	.0	.00
15	25.	0.	.0	.00
16	31.	0.	.0	.00
17	35.	0.	.0	.00
18	28.	0.	.0	.00
19	32.	0.	.0	.00
20	27.	0.	.0	.00
21	29.	0.	.0	.00
22	36.	0.	.0	.00
23	27.	0.	.0	.00
24	28.	0.	.0	.00
25	27.	0.	.0	.00
26	29.	0.	.0	.00

APPENDIX 3

(continued)

FIGURE 6

Elution from hplc column of faeces extracts 2 + 3 dog 1, 48-72 hours



APPENDIX 4

Hplc of plasma extracts

(Results expressed as dpm)

TABLE 1

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 1 HOUR

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 9.58 9.581%
TOTAL DPM ELUTED = 167

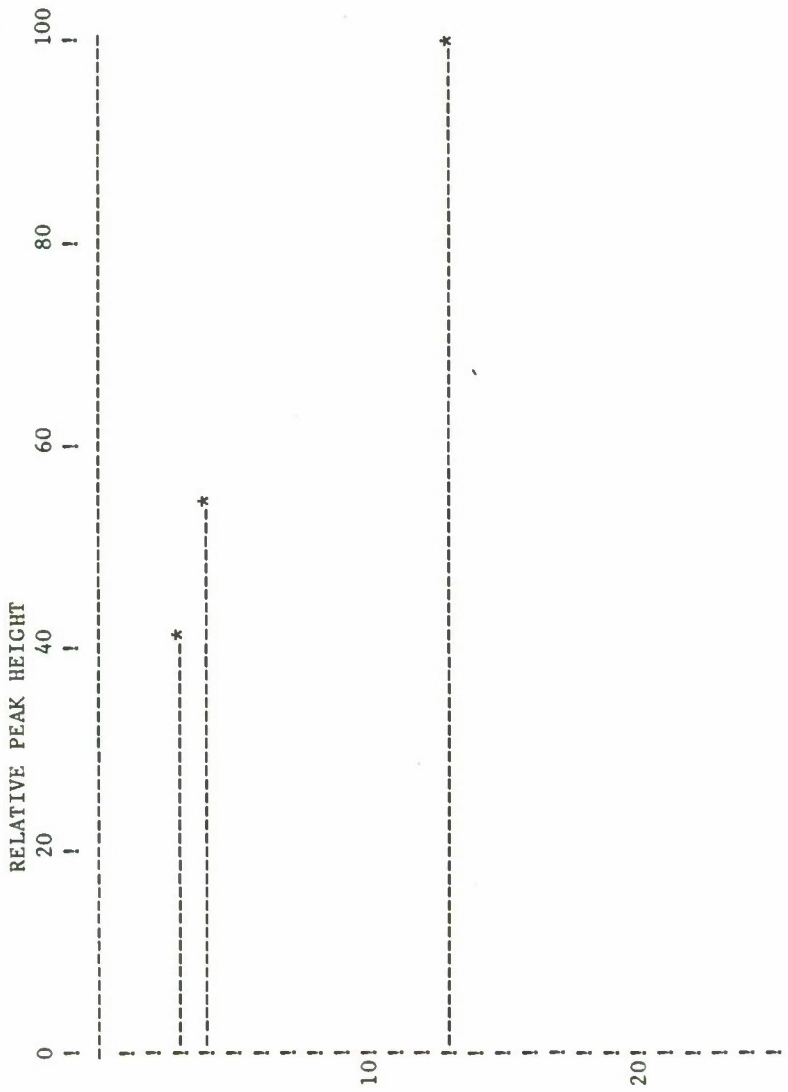
FRACTION(MINS)	GROSS	NET	%	%
1	41.	0.	.0	.00
2	42.	0.	.0	.00
3	79.	35.	21.0	20.96
4	91.	47.	28.1	28.14
5	58.	0.	.0	.00
6	47.	0.	.0	.00
7	45.	0.	.0	.00
8	40.	0.	.0	.00
9	39.	0.	.0	.00
10	44.	0.	.0	.00
11	41.	0.	.0	.00
12	41.	0.	.0	.00
13	129.	85.	50.9	50.90
14	45.	0.	.0	.00
15	38.	0.	.0	.00
16	41.	0.	.0	.00
17	40.	0.	.0	.00
18	40.	0.	.0	.00
19	43.	0.	.0	.00
20	38.	0.	.0	.00
21	40.	0.	.0	.00
22	44.	0.	.0	.00
23	40.	0.	.0	.00
24	41.	0.	.0	.00
25	41.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 1

Elution from hplc column of plasma dog 1 at 1 hour



APPENDIX 4

(continued)

TABLE 2

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 2 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 3.94 3.941%
TOTAL DPM ELUTED = 406

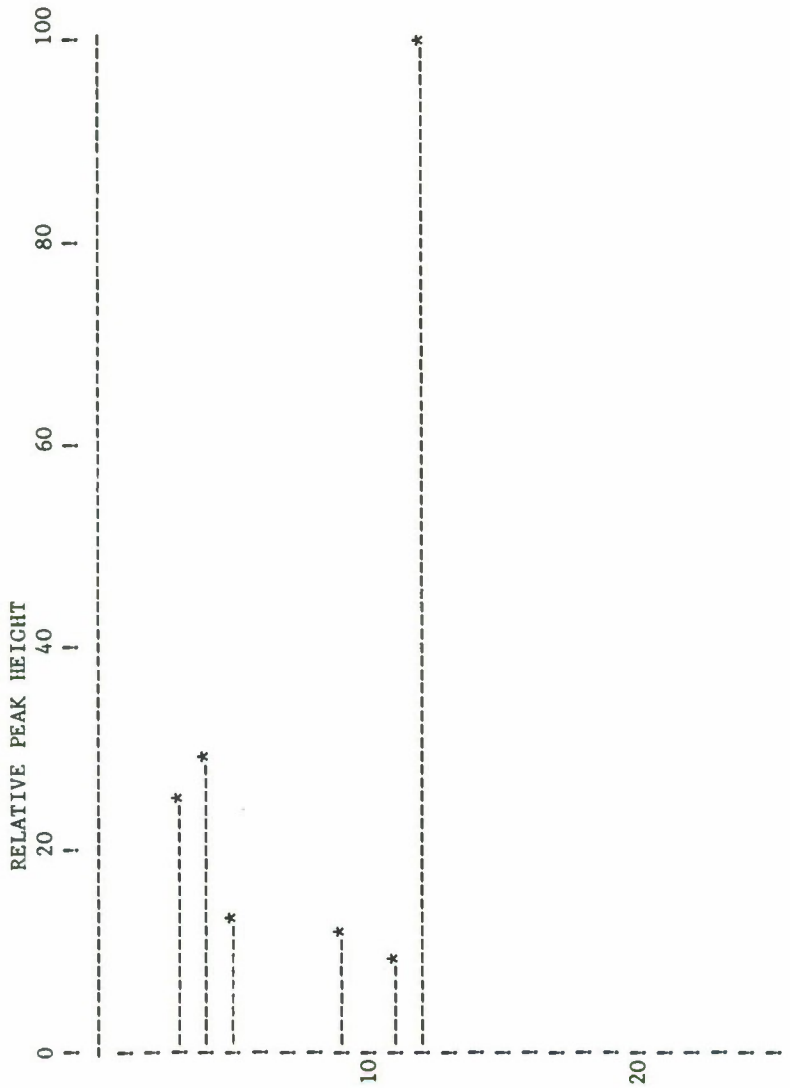
FRACTION(MINS)	GROSS	NET	%	%
1	45.	0.	.0	.00
2	48.	0.	.0	.00
3	99.	55.	13.5	13.55
4	107.	63.	15.5	15.52
5	73.	29.	7.1	7.14
6	51.	0.	.0	.00
7	46.	0.	.0	.00
8	47.	0.	.0	.00
9	69.	25.	6.2	6.16
10	49.	0.	.0	.00
11	63.	19.	4.7	4.68
12	259.	215.	53.0	52.96
13	54.	0.	.0	.00
14	45.	0.	.0	.00
15	45.	0.	.0	.00
16	46.	0.	.0	.00
17	45.	0.	.0	.00
18	43.	0.	.0	.00
19	38.	0.	.0	.00
20	48.	0.	.0	.00
21	43.	0.	.0	.00
22	44.	0.	.0	.00
23	48.	0.	.0	.00
24	45.	0.	.0	.00
25	42.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 2

Elution from hplc column of plasma dog 1 at 2 hours



APPENDIX 4

(continued)

TABLE 3

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 3 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 4.02 4.020%
TOTAL DPM ELUTED = 398

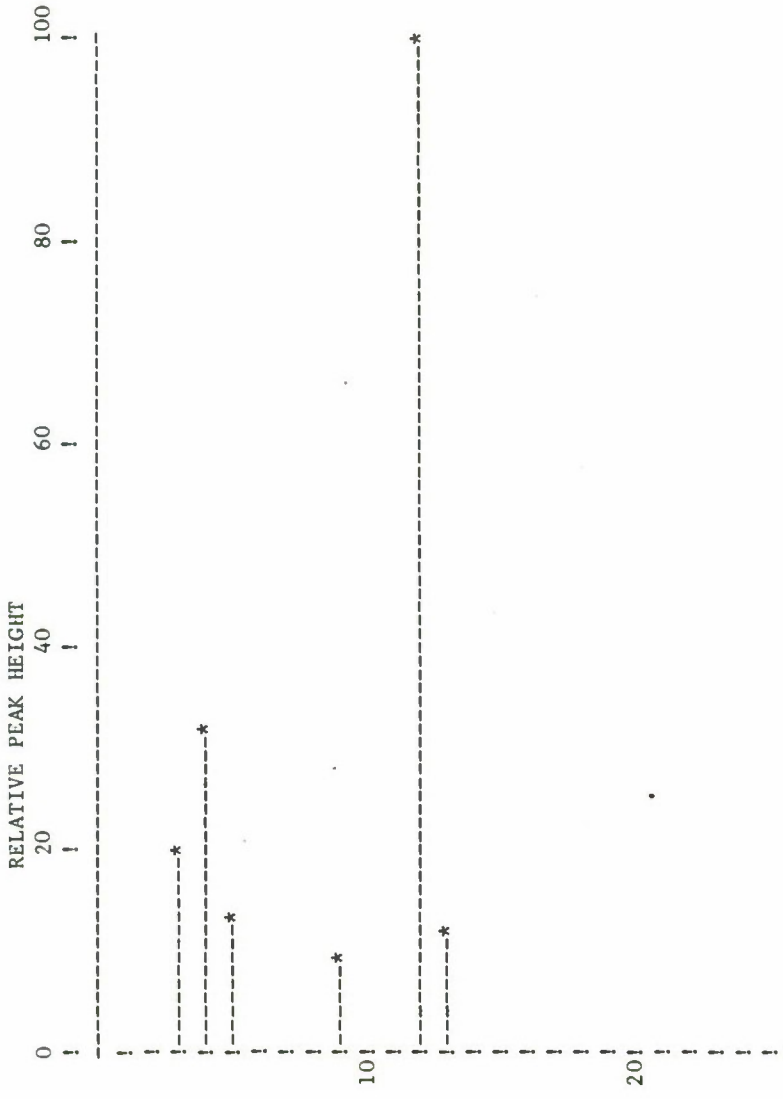
FRACTION(MINS)	GROSS	NET	%	%
1	55.	0.	.0	.00
2	46.	0.	.0	.00
3	87.	43.	10.8	10.80
4	112.	68.	17.1	17.09
5	73.	29.	7.3	7.29
6	48.	0.	.0	.00
7	44.	0.	.0	.00
8	47.	0.	.0	.00
9	65.	21.	5.3	5.28
10	49.	0.	.0	.00
11	49.	0.	.0	.00
12	255.	211.	53.0	53.02
13	70.	26.	6.5	6.53
14	46.	0.	.0	.00
15	45.	0.	.0	.00
16	38.	0.	.0	.00
17	42.	0.	.0	.00
18	47.	0.	.0	.00
19	39.	0.	.0	.00
20	43.	0.	.0	.00
21	42.	0.	.0	.00
22	44.	0.	.0	.00
23	43.	0.	.0	.00
24	46.	0.	.0	.00
25	45.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 3

Elution from hplc column of plasma dog 1 at 3 hours



APPENDIX 4

(continued)

TABLE 4

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 4 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 3.98 3.980%
TOTAL DPM ELUTED = 402

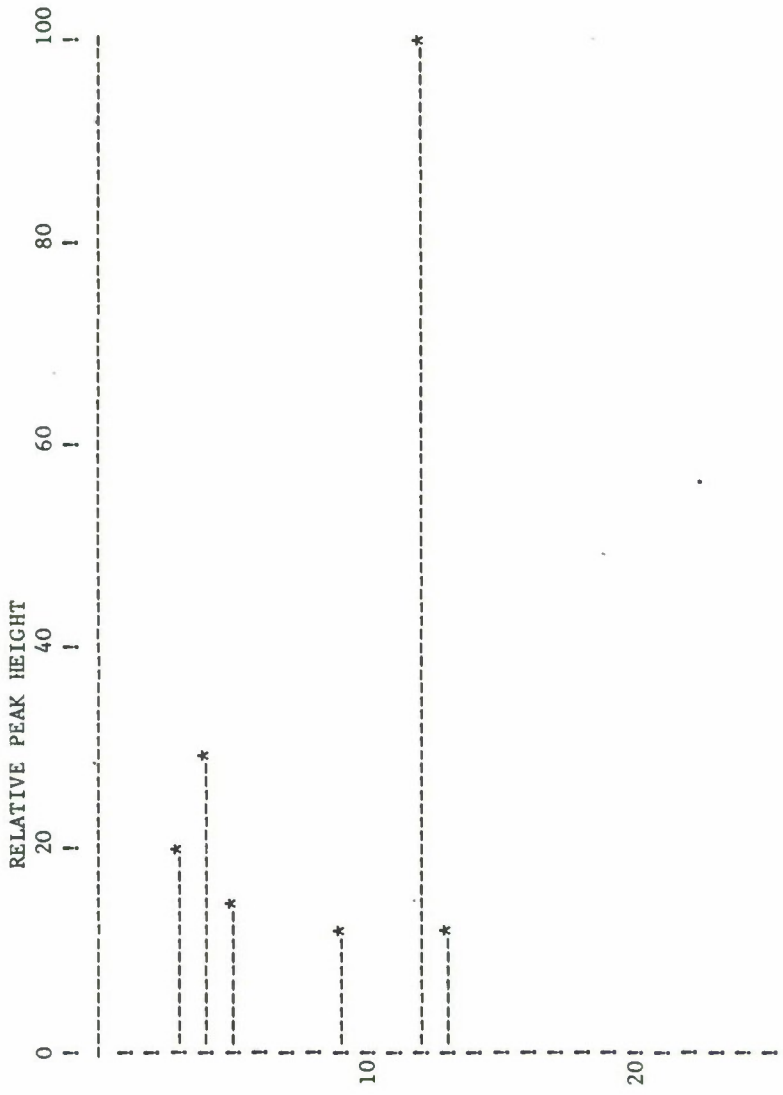
FRACTION(MINS)	GROSS	NET	%	%
1	47.	0.	.0	.00
2	47.	0.	.0	.00
3	86.	42.	10.4	10.45
4	108.	64.	15.9	15.92
5	76.	32.	8.0	7.96
6	52.	0.	.0	.00
7	48.	0.	.0	.00
8	48.	0.	.0	.00
9	69.	25.	6.2	6.22
10	43.	0.	.0	.00
11	47.	0.	.0	.00
12	258.	214.	53.2	53.23
13	69.	25.	6.2	6.22
14	49.	0.	.0	.00
15	50.	0.	.0	.00
16	45.	0.	.0	.00
17	45.	0.	.0	.00
18	47.	0.	.0	.00
19	44.	0.	.0	.00
20	45.	0.	.0	.00
21	40.	0.	.0	.00
22	44.	0.	.0	.00
23	42.	0.	.0	.00
24	48.	0.	.0	.00
25	41.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 4

Elution from hplc column of plasma dog 1 at 4 hours



(continued)

TABLE 5

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 5 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 4.03 4.030%
TOTAL DPM ELUTED = 397

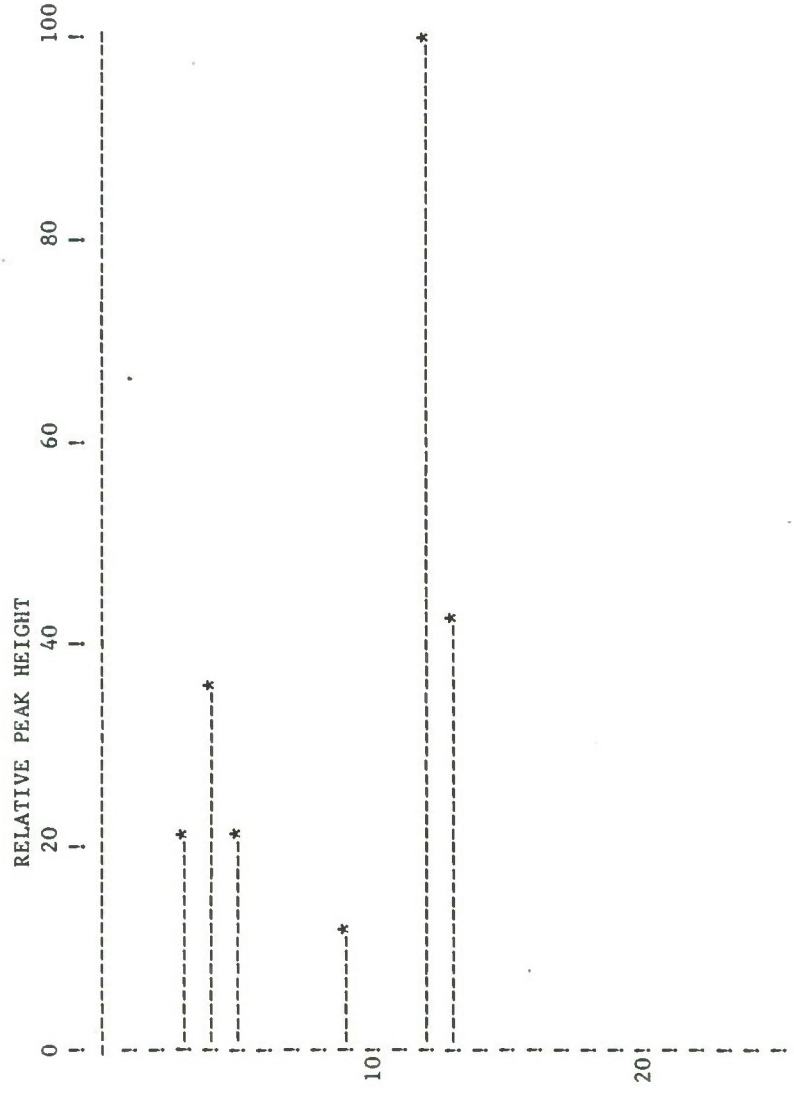
FRACTION(MINS)	GROSS	NET	%	%
1	46.	0.	.0	.00
2	47.	0.	.0	.00
3	80.	36.	9.1	9.07
4	106.	62.	15.6	15.62
5	81.	37.	9.3	9.32
6	57.	0.	.0	.00
7	50.	0.	.0	.00
8	49.	0.	.0	.00
9	64.	20.	5.0	5.04
10	56.	0.	.0	.00
11	44.	0.	.0	.00
12	214.	170.	42.8	42.82
13	116.	72.	18.1	18.14
14	46.	0.	.0	.00
15	48.	0.	.0	.00
16	47.	0.	.0	.00
17	47.	0.	.0	.00
18	44.	0.	.0	.00
19	43.	0.	.0	.00
20	42.	0.	.0	.00
21	44.	0.	.0	.00
22	46.	0.	.0	.00
23	44.	0.	.0	.00
24	45.	0.	.0	.00
25	41.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 5

Elution from hplc column of plasma dog 1 at 5 hours



(continued)

TABLE 6

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 6 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 4.55 4.545%
TOTAL DPM ELUTED = 352

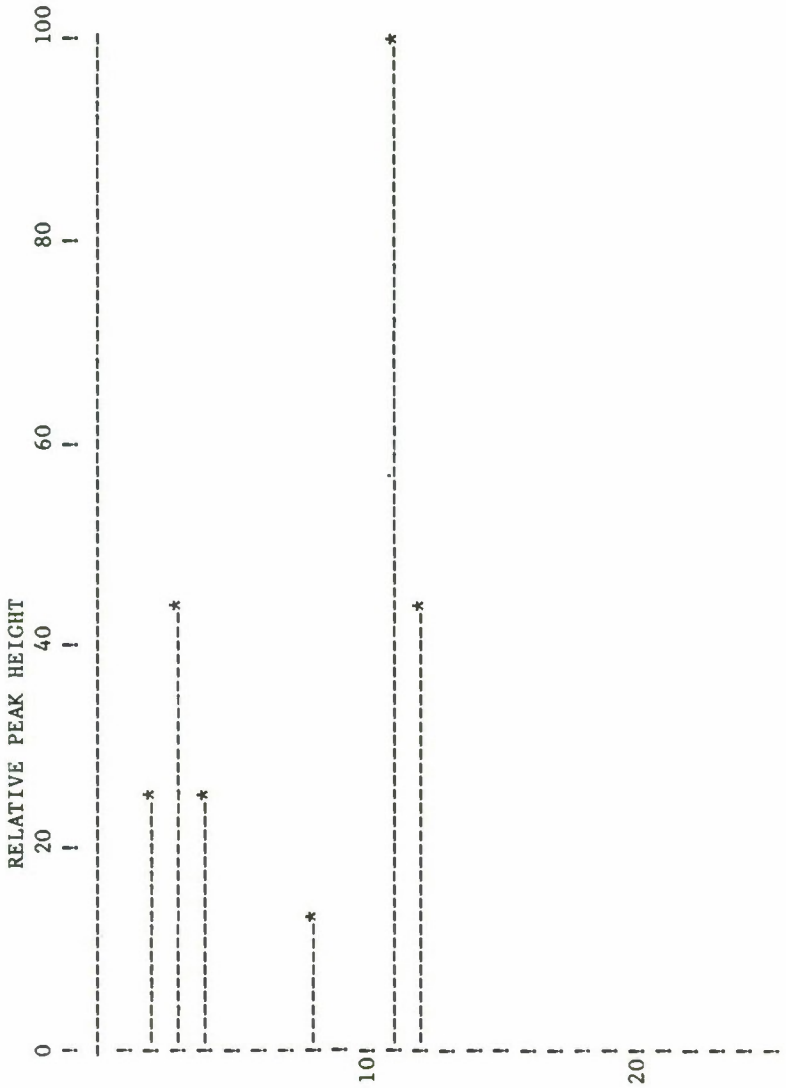
FRACTION(MINS)	GROSS	NET	%	%
1	48.	0.	.0	.00
2	79.	35.	9.9	9.94
3	106.	62.	17.6	17.61
4	80.	36.	10.2	10.23
5	52.	0.	.0	.00
6	47.	0.	.0	.00
7	48.	0.	.0	.00
8	62.	18.	5.1	5.11
9	55.	0.	.0	.00
10	43.	0.	.0	.00
11	183.	139.	39.5	39.49
12	106.	62.	17.6	17.61
13	51.	0.	.0	.00
14	48.	0.	.0	.00
15	47.	0.	.0	.00
16	46.	0.	.0	.00
17	43.	0.	.0	.00
18	45.	0.	.0	.00
19	45.	0.	.0	.00
20	49.	0.	.0	.00
21	46.	0.	.0	.00
22	48.	0.	.0	.00
23	42.	0.	.0	.00
24	44.	0.	.0	.00
25	45.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 6

Elution from hplc column of plasma dog 1 at 6 hours



APPENDIX 4

(continued)

TABLE 7

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 7 HOURS

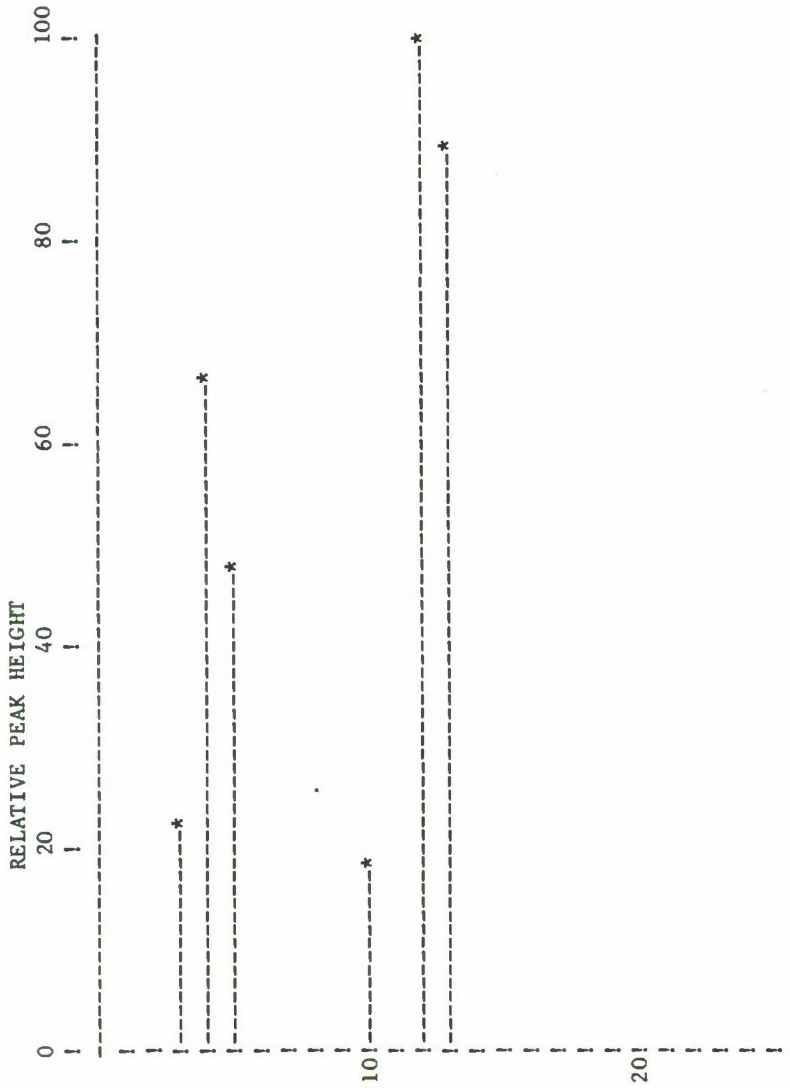
BACKGROUND = 44;LIMIT OF DETECTION = 16.0 4.68 4.678%
TOTAL DPM ELUTED = 342

FRACTION(MINS)	GROSS	NET	%	%
1	40.	0.	.0	.00
2	46.	0.	.0	.00
3	66.	22.	6.4	6.43
4	110.	66.	19.3	19.30
5	91.	47.	13.7	13.74
6	51.	0.	.0	.00
7	43.	0.	.0	.00
8	47.	0.	.0	.00
9	58.	0.	.0	.00
10	63.	19.	5.6	5.56
11	44.	0.	.0	.00
12	143.	99.	28.9	28.95
13	133.	89.	26.0	26.02
14	44.	0.	.0	.00
15	47.	0.	.0	.00
16	42.	0.	.0	.00
17	37.	0.	.0	.00
18	41.	0.	.0	.00
19	41.	0.	.0	.00
20	45.	0.	.0	.00
21	40.	0.	.0	.00
22	41.	0.	.0	.00
23	36.	0.	.0	.00
24	47.	0.	.0	.00
25	41.	0.	.0	.00

(continued)

FIGURE 7

Elution from hplc column of plasma dog 1 at 7 hours



APPENDIX 4

(continued)

TABLE 8

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 12 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 5.08 5.079%
TOTAL DPM ELUTED = 315

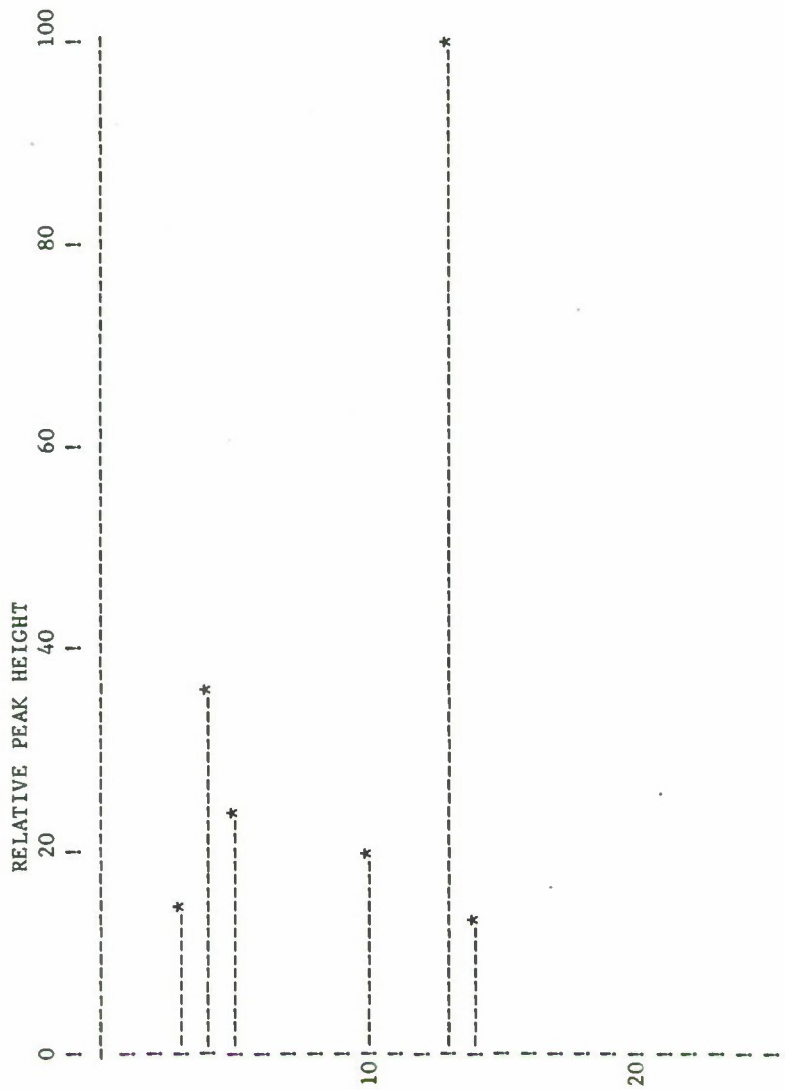
FRACTION(MINS)	GROSS	NET	%	%
1	40.	0.	.0	.00
2	44.	0.	.0	.00
3	66.	22.	7.0	6.98
4	99.	55.	17.5	17.46
5	80.	36.	11.4	11.43
6	55.	0.	.0	.00
7	48.	0.	.0	.00
8	42.	0.	.0	.00
9	45.	0.	.0	.00
10	74.	30.	9.5	9.52
11	44.	0.	.0	.00
12	45.	0.	.0	.00
13	196.	152.	48.3	48.25
14	64.	20.	6.3	6.35
15	41.	0.	.0	.00
16	39.	0.	.0	.00
17	48.	0.	.0	.00
18	43.	0.	.0	.00
19	43.	0.	.0	.00
20	41.	0.	.0	.00
21	36.	0.	.0	.00
22	38.	0.	.0	.00
23	44.	0.	.0	.00
24	47.	0.	.0	.00
25	40.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 8

Elution from hplc column of plasma dog 1 at 12 hours



APPENDIX 4

(continued)

TABLE 9

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 24 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 4.26 4.255%
TOTAL DPM ELUTED = 376

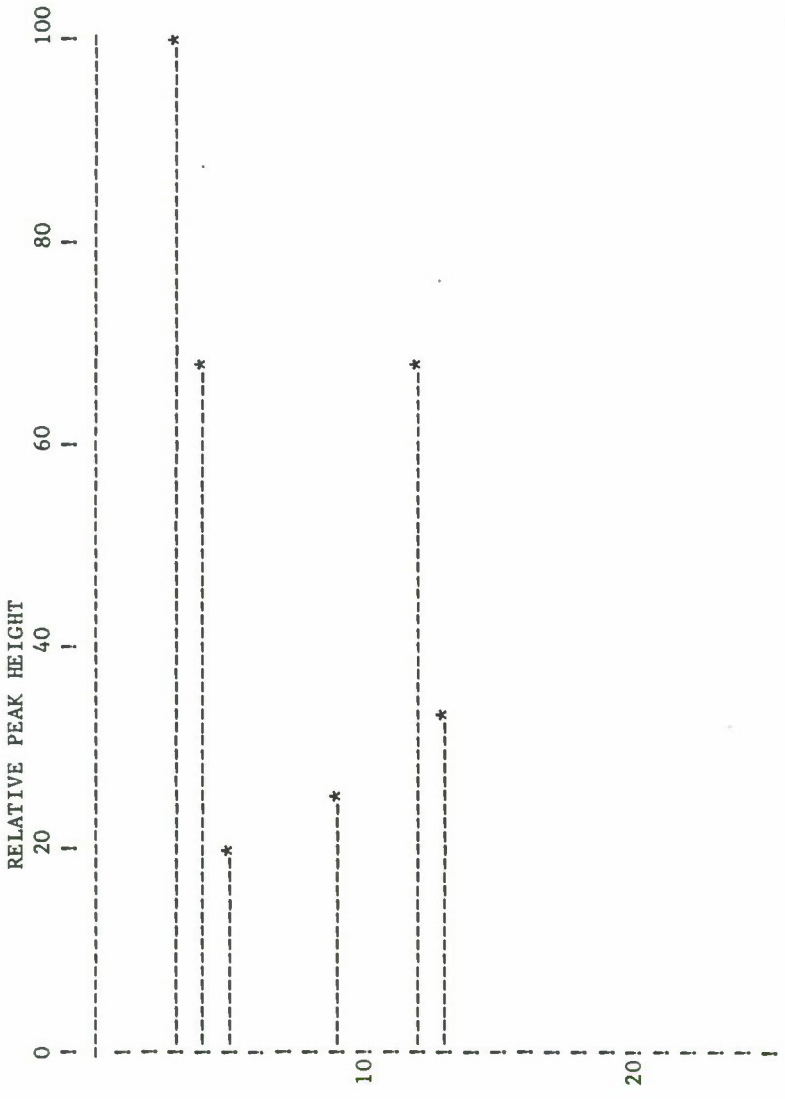
FRACTION(MINS)	GROSS	NET	%	%
1	39.	0.	.0	.00
2	57.	0.	.0	.00
3	164.	120.	31.9	31.91
4	125.	81.	21.5	21.54
5	68.	24.	6.4	6.38
6	44.	0.	.0	.00
7	42.	0.	.0	.00
8	49.	0.	.0	.00
9	74.	30.	8.0	7.98
10	52.	0.	.0	.00
11	42.	0.	.0	.00
12	125.	81.	21.5	21.54
13	84.	40.	10.6	10.64
14	41.	0.	.0	.00
15	43.	0.	.0	.00
16	42.	0.	.0	.00
17	43.	0.	.0	.00
18	42.	0.	.0	.00
19	41.	0.	.0	.00
20	44.	0.	.0	.00
21	40.	0.	.0	.00
22	39.	0.	.0	.00
23	46.	0.	.0	.00
24	43.	0.	.0	.00
25	40.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 9

Elution from hplc column of plasma dog 1 at 24 hours



APPENDIX 4

(continued)

TABLE 10

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 30 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 4.60 4.598%
TOTAL DPM ELUTED = 348

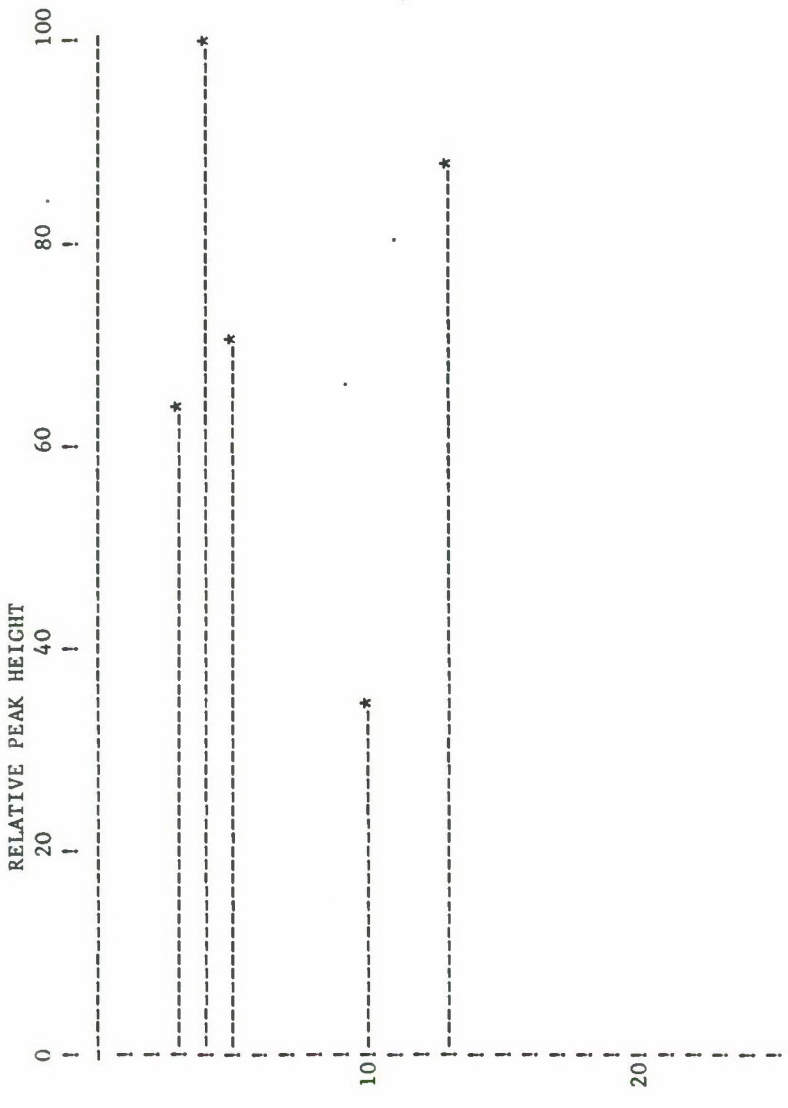
FRACTION(MINS)	GROSS	NET	%	%
1	44.	0.	.0	.00
2	43.	0.	.0	.00
3	106.	62.	17.8	17.82
4	141.	97.	27.9	27.87
5	113.	69.	19.8	19.83
6	51.	0.	.0	.00
7	42.	0.	.0	.00
8	41.	0.	.0	.00
9	51.	0.	.0	.00
10	78.	34.	9.8	9.77
11	46.	0.	.0	.00
12	42.	0.	.0	.00
13	130.	86.	24.7	24.71
14	55.	0.	.0	.00
15	45.	0.	.0	.00
16	44.	0.	.0	.00
17	40.	0.	.0	.00
18	38.	0.	.0	.00
19	40.	0.	.0	.00
20	43.	0.	.0	.00
21	40.	0.	.0	.00
22	42.	0.	.0	.00
23	38.	0.	.0	.00
24	39.	0.	.0	.00
25	42.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 10

Elution from hplc column of plasma dog 1 at 30 hours



APPENDIX 4

(continued)

TABLE 11

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 48 HOURS

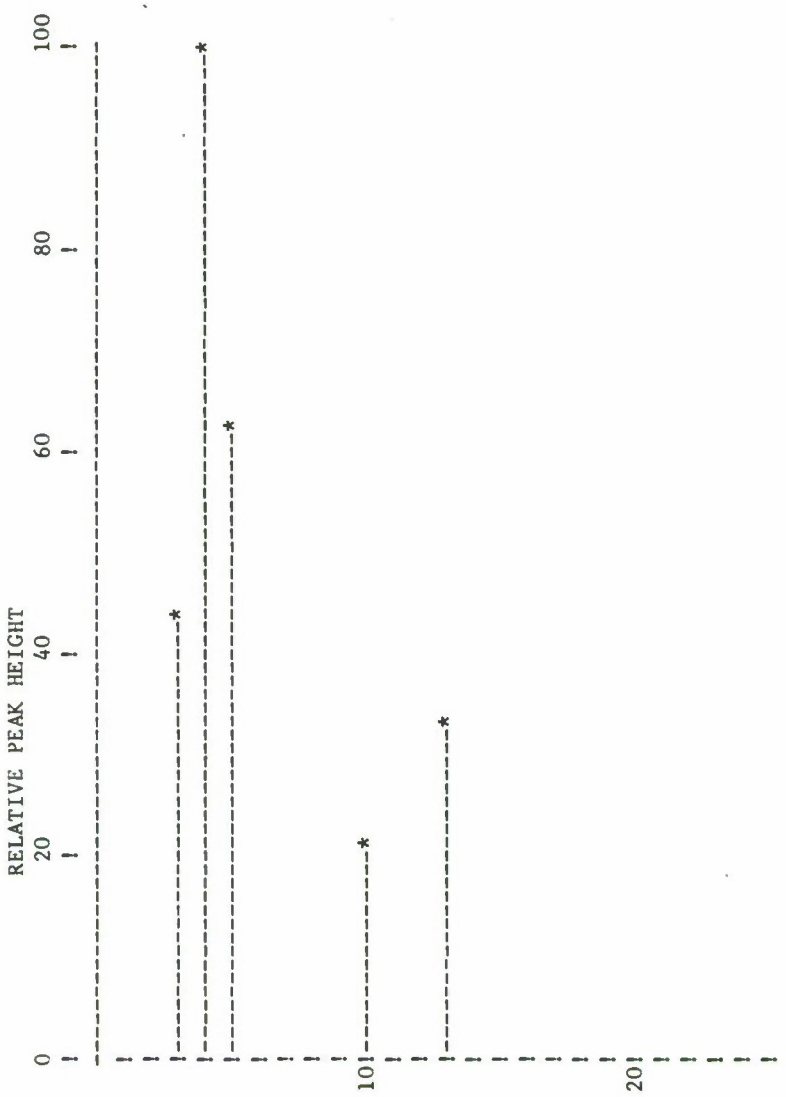
BACKGROUND = 44;LIMIT OF DETECTION = 16.0 5.03 5.031%
TOTAL DPM ELUTED = 318

FRACTION(MINS)	GROSS	NET	%	%
1	39.	0.	.0	.00
2	42.	0.	.0	.00
3	98.	54.	17.0	16.98
4	165.	121.	38.1	38.05
5	120.	76.	23.9	23.90
6	51.	0.	.0	.00
7	44.	0.	.0	.00
8	42.	0.	.0	.00
9	47.	0.	.0	.00
10	70.	26.	8.2	8.18
11	44.	0.	.0	.00
12	48.	0.	.0	.00
13	85.	41.	12.9	12.89
14	48.	0.	.0	.00
15	39.	0.	.0	.00
16	42.	0.	.0	.00
17	41.	0.	.0	.00
18	42.	0.	.0	.00
19	44.	0.	.0	.00
20	42.	0.	.0	.00
21	42.	0.	.0	.00
22	43.	0.	.0	.00
23	39.	0.	.0	.00
24	39.	0.	.0	.00
25	37.	0.	.0	.00

(continued)

FIGURE 11

Elution from hplc column of plasma dog 1 at 48 hours



APPENDIX 4

(continued)

TABLE 12

ELUTION FROM HPLC COLUMN OF PLASMA DOG 1 AT 72 HOURS

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 8.25 8.247%
TOTAL DPM ELUTED = 194

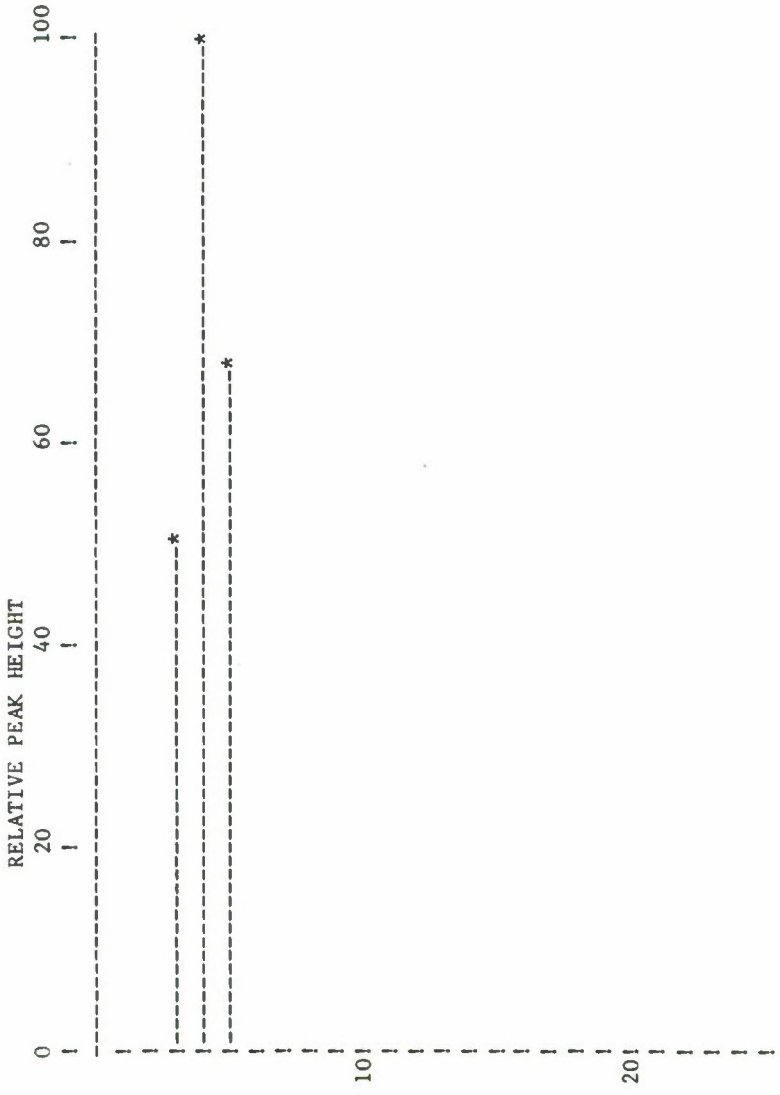
FRACTION(MINS)	GROSS	NET	%	%
1	45.	0.	.0	.00
2	41.	0.	.0	.00
3	89.	45.	23.2	23.20
4	133.	89.	45.9	45.88
5	104.	60.	30.9	30.93
6	45.	0.	.0	.00
7	42.	0.	.0	.00
8	38.	0.	.0	.00
9	42.	0.	.0	.00
10	58.	0.	.0	.00
11	45.	0.	.0	.00
12	40.	0.	.0	.00
13	56.	0.	.0	.00
14	47.	0.	.0	.00
15	41.	0.	.0	.00
16	43.	0.	.0	.00
17	43.	0.	.0	.00
18	42.	0.	.0	.00
19	42.	0.	.0	.00
20	40.	0.	.0	.00
21	39.	0.	.0	.00
22	45.	0.	.0	.00
23	42.	0.	.0	.00
24	41.	0.	.0	.00
25	41.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 12

Elution from hplc column of plasma dog 1 at 72 hours



APPENDIX 4

(continued)

TABLE 13

ELUTION FROM HPLC COLUMN OF SPIKED CONTROL PLASMA

BACKGROUND = 44;LIMIT OF DETECTION = 16.0 .10 .095%
TOTAL DPM ELUTED = 16829

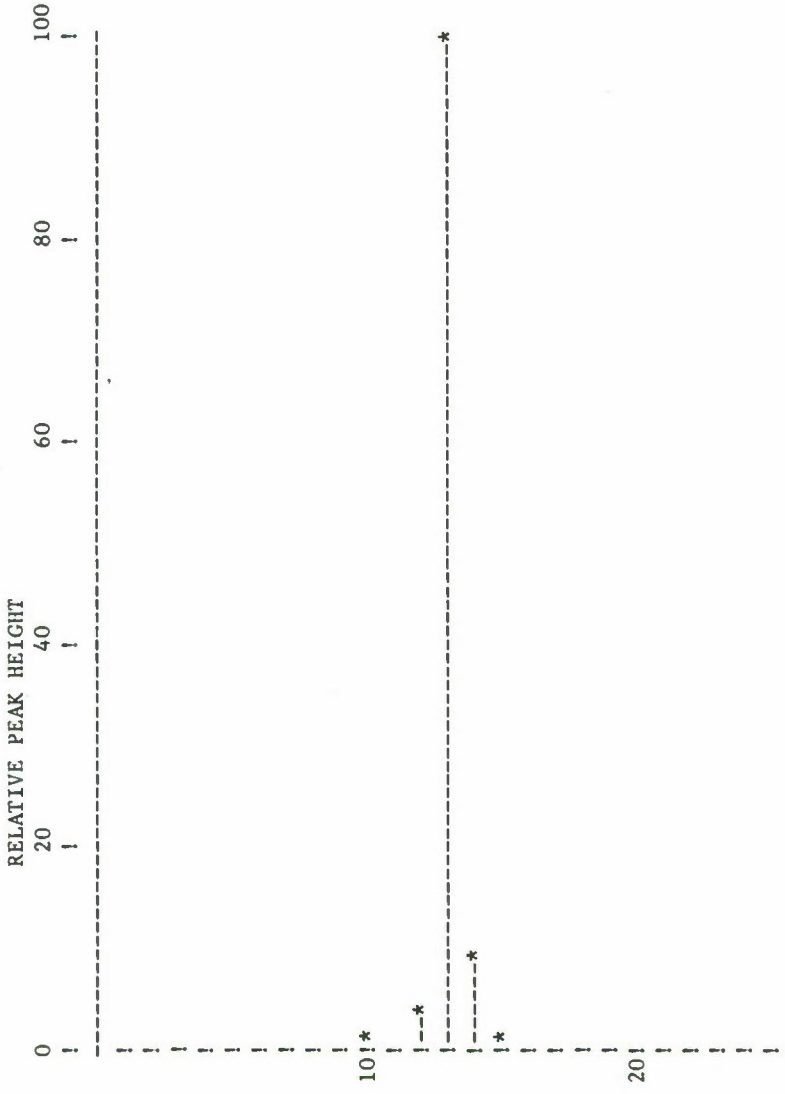
FRACTION(MINS)	GROSS	NET	%	%
1	44.	0.	.0	.00
2	39.	0.	.0	.00
3	60.	16.	.1	.10
4	76.	32.	.2	.19
5	52.	0.	.0	.00
6	54.	0.	.0	.00
7	52.	0.	.0	.00
8	87.	43.	.3	.26
9	98.	54.	.3	.32
10	176.	132.	.8	.78
11	95.	51.	.3	.30
12	589.	545.	3.2	3.24
13	14462.	14418.	85.7	85.67
14	1353.	1309.	7.8	7.78
15	171.	127.	.8	.75
16	121.	77.	.5	.46
17	69.	25.	.1	.15
18	52.	0.	.0	.00
19	52.	0.	.0	.00
20	48.	0.	.0	.00
21	52.	0.	.0	.00
22	46.	0.	.0	.00
23	46.	0.	.0	.00
24	44.	0.	.0	.00
25	47.	0.	.0	.00

APPENDIX 4

(continued)

FIGURE 13

Elution from hplc column of spiked control plasma



APPENDIX 5

Quantities of radioactivity in the excreta of a beagle dog following oral administration of ^{14}C-WR 178,460.HCl

Sample	Time (hours)	Sample volume (ml) or weight (g)	Radioactivity concentration (dpm/ml or dpm/g)
Urine	0- 6	NS	NS
	6- 24	365	490
	24- 48	405	228
	48- 72	395	174
	72- 96	370	99
	96-120	310	91
	120-144	255	98
	144-168	375	37
Cage wash	0- 24	1118	687
	24- 48	1114	404
	48- 72	1120	401
	72- 96	1140	295
	96-120	1160	379
	120-144	1010	113
	144-168	1180	38
Faeces extracts	0- 24	700	95163
		590	144819
		625	93585
	24- 48	510	12171
		445	16849
		435	10795
	48- 72	450	5277
		460	17911
		400	11776
	72- 96	620	4678
		610	10843
		640	5007
	96-120	340	5023
		340	6542
		315	3723
	120-144	370	2940
		365	2831
		340	2169
	144-168	660	1410
		570	1461
		570	883
Faeces residue	0- 24	369.6	186039
	24- 48	277.2	50837
	48- 72	308.1	40425
	72- 96	202.1	31556
	96-120	185.4	15200
	120-144	152.6	11992
	144-168	407.8	3754

NS No sample

Concentrations of radioactivity in plasma and whole-blood of a beagle dog after oral administration of ^{14}C-WR 178,460.HCl

Results expressed as dpm

Time	Plasma (dpm/ml)	Whole-blood (dpm/g)
15 min	103	106
30 min	314	276
1 hr	466	428
2 hrs	1012	785
3 hrs	1040	932
4 hrs	1068	982
5 hrs	1041	1038
6 hrs	1010	1036
7 hrs	968	1052
12 hrs	916	1002
24 hrs	1121	1076
30 hrs	1149	1054
2 days	1036	855
3 days	763	461
4 days	479	354
5 days	317	205
6 days	213	118
7 days	154	100
8 days	140	<90
10 days	85	<90
12 days	74	<90
14 days	64	<90
18 days	50	<90
21 days	35	<90

APPENDIX 7

Observed and fitted values of total radioactivity concentrations in plasma, and residuals (difference between observed and fitted), for the triple exponential model

t (hours)	c (dpm/ml)		Residual
	Observed	Fitted	
0.25	103	106	- 3
0.5	314	269	45
1	466	527	- 61
2	1012	848	164
3	1040	1014	26
4	1068	1096	- 28
5	1041	1133	- 92
6	1010	1146	-136
7	968	1147	-179
12	916	1092	-176
24	1121	941	180
30	1149	873	276
48	1036	699	337
72	763	523	240
96	479	394	85
120	317	300	17
144	213	231	- 18
168	154	181	- 27
192	140	144	- 4
240	85	97	- 12
288	74	71	3
336	64	57	7
432	50	43	7
504	35	39	- 4

APPENDIX 8

Observed and fitted values for the concentration of unchanged WR 178,460 in plasma, and residuals, for the double exponential model with two different time lags

Time (hours)	Observed c (μg/ml)	τ = 0.724h		τ = 0.107h	
		Fitted	Residual	Fitted	Residual
1	0.15	0.15	0.00	0.19	-0.04
2	0.37	0.37	0.00	0.31	0.06
3	0.39	0.40	-0.01	0.36	0.03
4	0.40	0.40	0.00	0.39	0.01
5	0.40	0.39	0.01	0.39	0.01
6	0.37	0.38	-0.01	0.39	-0.02
7	0.34	0.36	-0.02	0.38	-0.04
12	0.32	0.31	0.01	0.32	-0.00
24	0.23	0.21	0.02	0.21	0.02
30	0.18	0.17	0.01	0.17	0.01
48	0.08	0.09	-0.01	0.09	0.01
72	< 0.04+	0.04	-	0.04	-

+ Not used in fitting